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EDITORIAL COMMITTEE

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ERRATA

Page 108, footnote, "no. 1, p. 1–25, 1 fig." should read "no. 1, p. 1–25, 1912."

Page 111, line 7 from bottom, "Protein bodies stain," etc., should read "These so-called protein bodies stain," etc.

Page 172, Table I, last column, line 13, "9" should read "0."

Page 230, line 12, "methylene violet and with eosin" should read "methylene violet with eosin."

Page 234, line 6, "indicating presence of a reagent" should read "indicating the presence of the reagent."

Page 279 "*Rheosporangium aphanidermatus*" should read "*Rheosporangium aphanidermatum*.

Page 291, line 7, "*Rheosporangium aphanidermatus* n. sp." should read "*Rheosporangium aphanidermatum*, n. sp."

Page 306, line 2 from bottom, "which were treated as noted before" should read "which were treated as hereafter noted."

Page 344, line 10, "as indicated under 1" should read "as indicated under a."

Page 368, line 2 from bottom, "A new method of enumerating bacteria" should read "A new method of enumerating bacteria in air."

Page 455, legend under figure 3, "center" should read "outer."

Page 477, line 18 from top, "It is Gram-positive" should read "It is Gram-negative."

Page 477, line 23 from top, "*Bacillus lactuacae*" should read "*Bacillus lactucae*."

ILLUSTRATIONS

PLATES

RHEOSPORANGIUM APHANIDERMATUM, A NEW GENUS AND SPECIES OF FUNGUS PARASITIC ON SUGAR BEETS AND RADISHES

PHOMA DESTRUCTIVA, THE CAUSE OF A FRUIT ROT OF THE TOMATO

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OCCURRENCE AND GENERAL APPEARANCE OF THE DISEASE

In March, 1912, specimens of tomatoes (*Lycopersicon esculentum*) affected with a fruit rot were sent in for examination from Cutler, Fla. Mr. James B. Brown, who selected and sent in the specimens, stated in an accompanying letter that great loss had been caused among the farmers of Dade County, Fla., by this fruit-spotting. When received, some of the fruit was green, some ripe, and some just beginning to color. Most of the tomatoes had conspicuous dark spots on the side and at the stem end. These spots, occurring on both green and ripe fruit and measuring 1 to 3 cm. in diameter, were brownish black in color, with definite outlines, while on the surface of the largest spots tiny dark pustules could be seen. The firm, discolored tissue of the spots was somewhat sunken, forming slight depressions, the surface of which was membranous or crustlike, according to the stage of development. On the ripe fruit the dark tissue was surrounded by a more or less watery-looking zone. Plate A, figure 1, shows the fruit-spotting as it appeared upon one of the Cutler specimens.

A microscopic examination of tissue cut from diseased areas occurring on both green and ripe tomatoes showed a dense network of fungous mycelium within the cells. The hyphæ were septate, branched, and hyaline to brown in color, while scattered over the surface of the mycelial growth numerous small dark pycnidia could be seen (Pl. I, fig. 1). These pycnidia, varying considerably in size, were for the most part round in outline, with a distinct central pore, out of which issued masses of hyaline 1-celled spores in long coils. The structure of a pycnidium with its relation to the host cells is shown in Plate I, figure 1, and a few single hyphal strands in Plate I, figure 2. Bacteria were also numerous in the tissue of the diseased area, and in some of the spots spores of

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(1)

Macrosporium were observed. Since, however, the pycnidial fungus, whose general appearance indicated that it belonged to the genus Phoma, was found in such abundance in tissue from all of the spots examined, it seemed probable that this fungus might prove to be the primary cause of this tomato fruit-rot. An isolation of this fungus from diseased tissue was therefore undertaken.

ISOLATION OF THE FUNGUS FROM DISEASED TOMATO FRUIT

With sterilized instruments small pieces of diseased tissue, partially sterilized in a solution of mercuric chlorid (1 to 1,000), were cut out from beneath the surface of several of the spots and transferred to cultural media. A grayish white mycelial growth developed on string-bean agar, and at the end of two weeks small dark pycnidia similar to those found on the tomato fruit were observed. Under the microscope numerous hyaline 1-celled vacuolated spores could be seen issuing from the crushed pycnidia. Some of these spores were transferred to sterilized potato cylinders, cotton and tomato stems, lima-bean, prune, and string-bean agars, and from these transfers pure cultures of the Phoma fungus were obtained and later used in inoculation experiments. Pycnidia and spores were produced in abundance on potato cylinders, tomato stems, and upon lima-bean and prune-agar slants.

Nearly two years after the receipt of the Cutler specimens some diseased tomatoes affected with a similar spotting were sent in (February, 1914) by A. F. Young & Co., New York City. These tomatoes were selected from shipments received from Cuba (Pl. III, fig. 1) and from Punta Gorda, Fla. By the usual agar plate methods a Phoma fungus, identical with that isolated from the Cutler fruit, was obtained from tissue cut from spots occurring upon specimens from both Cuba and Florida.

INOCULATION EXPERIMENTS

INFECTION OF GREEN AND RIPE FRUIT IN THE GREENHOUSE

Inoculation experiments were begun on March 30, 1912, with some large tomato plants growing in the greenhouse. Needle-prick inoculations were made into green and ripening fruit, and also into the stems and leaves of these plants, the Phoma fungus being transferred from 20-day-old cultures of string-bean agar. Plants having fruit, stem, and leaves pricked with a sterilized needle were used as controls. In four to six days spots developed on green and ripe tomatoes, occurring upon the sides, stem end, and blossom end of the fruit, according to the position of the inoculation pricks. The spots thus produced by inoculation of the fungus were similar in appearance to those observed upon the diseased specimens from Florida. A week after inoculation the stem tissue of the tomato plant was seen to be slightly discolored about the needle pricks. This discoloration, however, had not spread to any considerable extent, and the surrounding tissue still remained

firm. Cross sections of a stem cut through the point of inoculation showed a brownish area, with mycelium in the epidermal and outer vascular tissue. On the leaves no evidence of spotting was observed two to four weeks after inoculation.[1] At the end of two weeks the spots produced on the fruit by inoculation had spread considerably beyond the needle pricks, and the tissue had perceptibly darkened. The ripe tomatoes were beginning to fall from the vines. Some of these inoculated specimens were brought to the laboratory for examination. The almost black spots, measuring 2 to 3.5 cm. in diameter on the ripe fruit, were surrounded by a lighter zone the tissue of which was soft and somewhat sunken, while that of the central portion of the spot was black and crustlike. Tissue from the discolored areas was examined and found to be full of fungus mycelium, while on the surface of the crustlike portion numerous dark pycnidia could be seen. The hyaline to brownish hyphæ were septate and branched, and the dark spherical pycnidia contained masses of 1-celled hyaline spores which issued in coils from a central pore. A microscopic study of this fungus, made from diseased tissue taken from inoculated fruit spots, showed it to be the same as that used in the inoculation and previously isolated from the Cutler material.

The spots produced on the green fruit two weeks after inoculation were not as large as those of the ripe tomatoes, measuring only 1 to 1.5 cm. in diameter, and the discoloration presented a somewhat different appearance. The central portion of the spot on the green fruit was of lighter color, becoming darker toward the circumference and merging into a definite dark-brown border.

A second inoculation experiment with greenhouse tomato plants was begun on April 17, 1912, when green and partly ripened fruits were inoculated by means of needle pricks with fungus from 22-day-old tomato-stem cultures. Two weeks after inoculation conspicuous dark diseased areas had developed, similar in appearance to the spots produced by the previous inoculation and to those of the diseased Florida tomatoes. Examination of the darkened tissue showed mycelium, pycnidia, and spores of the fungus inoculated.

An interruption of the work occurred at this time, and further study of this tomato fruit rot was not resumed until the spring of 1913. Cultures of the Phoma fungus were kept growing upon different kinds of media, transfers being made every 8 to 10 weeks. In February, 1913, the work was resumed. Questions as to how early the fungus is able to infect the fruit and the means by which it gains entrance to the tissue suggested themselves, and experiments in greenhouse and laboratory were begun in order to determine something definite in regard to these points.

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[1] Later experiments proved the fungus to be parasitic under certain conditions of moisture and temperature upon both leaves and stems of the tomato. Failure to produce the disease in the early experiments is believed to have been due to the low humidity and temperature of the atmosphere of the greenhouse.

Twelve young tomato plants, 30 to 40 cm. high, blossoming and with fruit set, were selected for one experiment. The plants were growing in pots in the greenhouse and were in a healthy condition.

A fungous suspension was made by pouring 10 c. c. of sterilized water into 53-day-old cotton-stem cultures of the fungus which were rich in pycnidia and spores. The culture tubes were well shaken, after which the suspension was poured off into clean tubes.

Three methods of inoculation were used:

(1) The spore suspension was transferred by means of a camel's-hair brush to the fruit of two plants. Care was taken not to injure the tissue of one plant while needle pricks were made into the fruit of the other.

(2) By means of an atomizer the suspension was sprayed upon four plants, thoroughly wetting the fruit. Two of these plants were not wounded and two were injured after spraying by pricking the fruit with a sterilized needle.

(3) Direct needle-prick inoculations from cotton-stem cultures of the fungus were made into the fruit of three plants.

Three plants having fruit pricked with a sterilized needle were set aside as controls. During the first 24 hours these 12 plants were protected from sunlight by strips of thick manila paper. Observations were made every few days and records taken at the end of 6, 12, 24, and 28 days. The results are given in Table I.

TABLE I.—*Results of inoculations of young green tomato fruit with Phoma fungus (greenhouse experiment)*

Method of inocu-lation.	Results of inoculation.			
	After 6 days.	After 12 days.	After 24 days.	After 28 days.
Spore suspension transferred with brush.	No infection of fruit not wound-ed. Slight dis-coloration of tis-sue upon needle-pricked green fruit.	No infection of fruit not wounded. Discoloration spreading about pricks on green fruit.	Tomatoes beginning to turn red. No infection of fruit not wounded. Definite spots forming on needle-pricked fruit.	Tissue of ripening tomatoes firm, with no evidence of infection of fruit not wound-ed. Diseased spots produced about needle pricks.
Spore suspension sprayed on fruit.	No infection of fruit not wound-ed. Discolora-tion of tissue on needle - pricked green fruit.	No infection of fruit not wounded. Discoloration spreading about inoculation pricks at stem end, blos-som end, and sides of green fruit.	No infection of fruit not wounded. Definite spots about needle pricks. Pycnidia forming on center of spots.	Tissue firm with no evidence of infec-tion on fruit not wounded. Dis-eased spots pro-duced about nee-dle pricks.
Inoculation by means of needle pricks.	Brownish black discoloration of tissue about in-oculation pricks on green tomato.	Definite brownish black spots devel-oped about all in-oculations on green fruit.	Spots 2 to 4 cm. in diameter, with black central por-tion, brownish cir-cumference, and watery band sur-rounding. Pyc-nidia scattered over central por-tion.	Conspicuous dis-eased spots pro-duced about nee-dle-prick inocu-lations.
Check plants. Fruit pricked with sterilized needle.	No infection.......	No infection.........	No infection	No infection.

Four weeks after inoculation the tomatoes which had been exposed without injury to the spraying of the spore suspension in the foregoing experiment were picked and placed in moist chambers in the laboratory. This was done in order to see whether infection would occur on the sprayed fruit when separated from the vine.   At the end of 12 days the tissue of these tomatoes remained firm and there was no evidence of infection.

Fully grown tomato plants bearing green and ripening fruit were used in another experiment in order to show the effect of fungous inoculations by the wounding and nonwounding of nearly mature tomatoes.   Transfers with a platinum loop were made from tomato-stem cultures of the Phoma fungus, to blossom end, stem end, and sides of green and ripening fruit.   Some of the tomatoes were needle-pricked and some were not injured.   Control plants were also used.   The observations given in Table II were made 5 and 10 days after inoculation.

TABLE II.—*Results of the inoculation of maturing tomato fruit with Phoma fungus* (*greenhouse experiment*)

Method of inoculation.	Results of inoculation.	
	After 5 days.	After 10 days.
Fungus transferred to surface of fruit. No wounding.	No discoloration at stem end, blossom end, or on sides of fruit.	No infection from inoculations.
Fungus transferred to surface of fruit, wounding by needle pricks.	Discoloration about needle pricks at stem end, blossom end, and sides of fruit.	Diseased spots produced about pricks with characteristic discoloration and decay.
Check tomatoes pricked with sterilized needle.	Free from infection.........................	Free from infection.

As a result of inoculations into young and maturing tomato fruits, it may be seen from data in Tables I and II that infection begins soon after the fruit sets, a slight discoloration or definite spotting developing within five to seven days, according to the method of inoculation used.   It is also apparent that the fungus is a wound parasite, since no infection occurred where the epidermis of the fruit remained unbroken.   Wounding of the fruit by insects, bruising, and natural cracking of the tissues no doubt affords a common means of entrance for this parasite.

In the inoculation work it was noticed that the discoloration of the tissue developed rapidly upon ripening tomatoes, the spots measuring 1 to 3 cm. in 10 days, while those upon the green fruit were only about one-fifth as large in the same length of time.   The discolored tissue of the ripe fruit varied from brown at the circumference to almost black in the central portion, the whole being bordered by a zone of watery-looking tissue.   In the green fruit a lighter central area became surrounded by a darker band, and the watery zone was not noticeable.   Numerous pycnidia developed upon the darkest tissue, giving the surface a pimply appearance.

In tomatoes diseased by inoculation with the Phoma fungus the discoloration was found to penetrate from 2 to 3 cm. into the interior of the fruit, and microscopic examination showed an abundance of hyaline to brownish mycelium in the watery-looking tissue, as well as in that of the darker central tissue.

INFECTION OF TOMATO FOLIAGE IN THE GREENHOUSE

As already mentioned in connection with the inoculation of tomato fruit, the Phoma fungus was also inoculated into both stem and leaf tissue of young and mature tomato plants growing in the greenhouse. In a few instances a slight discoloration had been noticed, but no definite spotting had been produced. Up to this time no effort had been made to subject the inoculated plants to a higher temperature or to a more humid atmosphere than that afforded by ordinary greenhouse conditions. In January, 1914, a series of experiments was begun in the greenhouse to determine whether or not leaf infection would occur in plants placed under conditions more favorable to the development of the fungus.

Healthy young tomato plants of the Earliana variety growing in pots in the greenhouse were used. When inoculated, the plants were from 10 to 15 cm. in height. A spore suspension of the Phoma fungus, made by adding sterile water to a corn-meal culture containing an abundance of mature pycnidia, was transferred by means of a platinum loop to the leaf surface. The tissue was then needle-pricked and the plants placed under glass bell jars. Control plants were used. The atmosphere beneath the jars soon became saturated, and drops of water collected on the inside of the jar and upon the surface of the plant. In three to five days a discoloration of the leaf tissue was noticed about the points of inoculation, and in eight days definite dark spots had developed. These spots varied in size from 2 to 10 mm. In some cases several spots had coalesced, spreading across the entire leaflet. Photographs were made of two of these diseased plants (Pl. II, figs. 3 and 4). A microscopical examination of tissue taken from these spots showed the mycelium, pycnidia, and spores of the Phoma. From diseased tissue cut from some of the spots agar plates were poured and the fungus reisolated in pure culture.

A second experiment with young tomato plants was tried, the inoculation being made by spraying with a spore suspension. No needle pricks were made. The plants were placed beneath glass bell jars in the greenhouse, and within five days dark spots had developed (Pl. B, figs. 2 and 3). Small drops of water were noticed on the plants, especially along the edges of the leaves. Frequently the infection started beneath these drops of water, the discoloration spreading from the edge of the leaf inward. Microscopical examination of tissue from the diseased areas showed mycelium and pycnidia of the Phoma fungus.

Control plants placed beneath bell jars and subjected to the same conditions of humidity and temperature as plants used in this and the former experiment gave no evidence of disease. Since young tomato plants had proved susceptible to infection of the fungus under conditions of high temperature and humidity, it was decided to subject mature tomato plants to a similar test. Accordingly two nearly mature tomato plants (90 to 100 cm. high) growing in pots and bearing green fruit were selected and placed in glass infection cages.[1] One of these plants was thoroughly sprayed (January 17, 1914) with a spore suspension of the fungus, made by adding distilled water to a 10-day-old corn-meal culture. Spraying was done with a small atomizer, the foliage, stem, and fruit being thoroughly wet with the solution. The other plant was placed in a similar cage and sprayed with sterile water. In four days small dark spots had appeared on the foliage of the inoculated plant, giving the lower leaves a speckled appearance. A diseased leaf was picked, brought to the laboratory, and examined. It was found that each dark spot represented a point of fungus infection, as many as 50 spots being counted on a single leaflet. By means of beef-agar plates pure cultures of the Phoma fungus were recovered from this diseased leaf tissue. Infection spread rapidly upon the plant in the cage, until in 10 days' time the whole plant was badly spotted. Six of the lowest leaves had fallen from the stem, while those a little higher up were discolored, shriveled, and drooping. The upper leaves which were still green showed dark, irregular spots. Upon some of the petioles dark streaks and blotches occurred. On January 27, 1914 (10 days after inoculation), a photograph was made of the diseased plant taken from the infection cage (Pl. II, fig. 2). The control plant which showed no spotting or falling away of leaves was also photographed (Pl. II, fig. 1). Some of the tissue from the diseased plant was again examined in the laboratory, and plate cultures were made. Out of tissue cut from leaf spots pure cultures were isolated. A few of the leaves which had fallen from the inoculated plant were photographed separately in order to show more clearly the character of the spotting (Pl. IV). Three weeks after inoculation green and partly ripened fruit growing upon this diseased plant showed no signs of disease.

INFECTION OF GREEN AND RIPE FRUIT IN THE LABORATORY

In addition to the greenhouse experiments some infection tests were carried on in the laboratory. Green and ripening tomatoes were picked from healthy plants growing in the greenhouse. This fruit was washed, soaked in mercuric-chlorid solution (1 to 1,000) for 30 minutes, and

[1] The infection cages used had glass tops and sides set in wooden frames. When placed over the inoculated plants, the whole cage was raised about 5 cm. by means of supports, in order to allow ventilation. Capacity of cage, 170,633 c. c.

placed in glass moist chambers. Transfers from a spore suspension of the Phoma fungus were made with a camel's-hair brush to the blossom end, the stem end, and the sides of the fruit, after which some of the tomatoes were needle-pricked, while care was taken not to wound the others. Check tomatoes not treated with the spore suspension but kept in moist chambers under similar conditions were used as controls. Four days after inoculation a darkening of the tissue became visible about the pricks, especially at the stem end of the tomatoes. Where there had been no wounding of the tissue, no discoloration appeared. Ten days after inoculation diseased areas 2 to 3 cm. in diameter had developed from needle-prick inoculations on the sides and at the stem end of the tomatoes. The dark tissue in the central part of the spots was surrounded by a watery-looking zone. Cracking of the discolored tissue had occurred in some cases, and within the cracks a grayish white mycelium could be seen. On tomatoes not wounded at the time of inoculation (and not cracking later) no discoloration appeared. There was, however, in some of the nonpricked tomatoes a cracking of the tissues at the stem end, and in these cases the typical discoloration due to the presence of the fungus was observed.

A final examination made at the end of three weeks showed well-developed diseased spots about needle-prick inoculations, while no spotting occurred upon uninjured tomatoes treated with the fungus, except where natural splitting of the tissue at the stem end allowed the fungus to enter. The check tomatoes showed no evidence of disease. Several of the needle-pricked tomatoes kept in the moist chamber and showing spots at the stem end of the fruit were selected and photographed. (Pl. A, figs. 2, 3, 4, and 5.) Freezing microtome sections were made from tissue taken from fruit spots and the development of pycnidia and pycnospores was studied. Plate I, figure 6, shows the structure of a pycnidium in cross section. Within the darker outer cells are several layers of hyaline cells from which arise the basidia bearing the spores. In Plate I, figure 3, a few of these spores are shown under higher magnification. Table III gives the results of these inoculations.

TABLE III.—*Results of the inoculation of green and ripe tomato fruit with Phoma fungus (laboratory experiment)*

Methods of inoculation.	Results of inoculation.		
	After 4 days.	After 10 days.	After 3 weeks.
Spore suspension transferred with brush to surface of fruit. Needle-pricked.	Darkening of tissue around pricks.	Distinct spots produced about pricks.	Characteristic spotting with pycnidia on the surface.
Spore suspension transferred with brush to surface of fruit. No wounding.	No discoloration of tissue.	No infection............	No infection, except at stem end, where splitting of tissue occurred.

A laboratory experiment was also tried under thermostatic conditions of temperature and moisture. Green tomatoes picked from plants growing in a garden plot near the pathological greenhouses were thoroughly washed in water, soaked in a 2 per cent formaldehyde solution for three hours, after which needle-prick inoculations were made (Aug. 1, 1914) from a 6-weeks-old culture of the Phoma fungus. The inoculated tomatoes were then wrapped separately in paraffin paper and placed in zinc boxes in different compartments of an incubator. Water in a lower tray kept the air saturated. Temperature records were taken each day and averaged for the period during which the experiment lasted. Uninoculated tomatoes were used as checks. At the end of 18 days the tomatoes kept at an average temperature of 6.6° C. showed no decay and only a slight discoloration of tissue about the needle pricks, while tomatoes kept at 19.7° C. showed decayed spots in every case. Within the tissue of these spots, measuring 2 to 4 cm. in diameter, pycnidia and spores of the Phoma were found in abundance.

INFECTION OF TOMATO PLANTS IN THE FIELD

On June 10, 1914, six tomato plants (Matchless variety) growing in garden plots at Arlington Farm were sprayed with a spore suspension of the Phoma fungus made from a corn-meal culture. On August 15 the plants were examined and a few spotted leaves picked and brought to the laboratory. Pycnidia and spores of a Phoma were found in tissue cut from the spots. A month later the plants were again examined, and the leaves, stem, and fruit were found to be affected. Material was collected and brought in for microscopical study. The brown discolored patches on the leaves had a grayish center, upon the surface of which scattered pycnidia could be seen. From these small brown pycnidia spores issued in coils. Within the discolored tissue of the petiole and stem similar pycnidia and spores were found. The spotting of the fruit was characteristic, and here, too, the Phoma fungus was found.

Experiments were also made on August 27, 1914, with six tomato plants of the Livingston Coreless variety. A spore suspension of the Phoma was used and the fruit was needle-pricked after spraying. On September 10 fruit spots were observed upon green, partly ripened, and fully ripened fruit. By means of agar plates the fungus was reisolated from fruit and stem tissue.

CROSS-INOCULATIONS BETWEEN TOMATO FRUIT AND LEAF

The foliage of young and nearly mature tomato plants was sprayed with a fungous suspension of the Phoma made from cultures isolated from diseased tomato fruit tissue. Diseased spots were produced in four to six days, and the fungus was recovered from the leaf tissue by means of agar plates. With cultures thus obtained needle-prick inoculations were made into green and ripening fruit (greenhouse plants), and the characteristic Phoma spotting was again produced.

OTHER PLANTS EXPOSED TO INFECTION OF THE FUNGUS

A number of experiments were made in order to test the pathogenicity of the Phoma fungus upon eggplant, potato, sugar beet, Jimson weed, garden pea, bean, and pepper plants.

EGGPLANT (*Solanum melongena*).—In February, 1914, young egg-plants growing in pots at the greenhouse were sprayed with a spore suspension of the Phoma fungus made from a 4-weeks-old corn-meal culture. Some of the sprayed plants were placed beneath glass bell jars, with cotton-plugged tops, and some were placed in a glass infection cage. Control plants were used in both cases. The bell jars were removed from a few of the plants after 48 hours, but were kept over others for a period of 10 days. At the end of this time numerous small brown spots had appeared on the leaves of the plants kept beneath the bell jars and upon the plants in the infection cage, but no spotting was found upon plants uncovered after 48 hours. Lower leaves of the diseased plants were yellowish and drooping and were the first to show the spotting. Tissue from these spots was examined with the microscope, and pycnidia and spores observed. Spores could be seen issuing from the pycnidia in long coils. The majority of spots remained small in size, only a few becoming 3 to 8 mm. in diameter. The spotted leaves did not fall from the stems, and the inoculated plants continued to grow and to produce new leaves. By means of agar plates the Phoma fungus was recovered from spotted tissue taken from plants kept under bell jars and from sprayed plants in the infection cage. Control plants kept under similar conditions of temperature and moisture showed no spotting. From these experiments it appears that the Phoma isolated from diseased tomato tissue is slightly pathogenic to eggplant,[1] but since spotting occurred only upon plants kept beneath bell jars or within the infection cage for several days, it is not probable that eggplants grown under ordinary conditions of temperature and moisture would become infected with this fungus.

POTATO PLANTS (*Solanum tuberosum*).—Healthy young potato[2] plants 15 to 20 cm. high, growing in pots at the greenhouse, were sprayed on January 28, 1914, with a spore suspension of the Phoma fungus made from a 2-weeks-old corn-meal culture. Some of the plants were covered with bell jars, while others were placed in an infection cage. The bell jars were removed from some of the plants after 48 hours, but were kept over others for a period of 10 days. Within six days discolored spots were noticed upon leaves of plants under the bell jars and upon leaves of those in the infection cages (Pl. B, fig. 1). The lowest leaves showed the

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[1] Cultures of the *Phoma* were compared with *Phomopsis vexans* (Sacc. and Syd.) Harter, recently described by L. L. Harter (1914) as causing fruit-rot, leaf-spot, and stem-blight of eggplant. The two fungi were found to be quite distinct morphologically, and according to inoculations made by Harter *Phomopsis vexans* is not parasitic on tomato.

[2] Saccardo reports the following species of Phoma on *Solanum tuberosum*: "*Phoma nebulosa* (Pers.) Mont." (1884, p. 135); "*Phoma eupyrena*" (1884, p. 127); "*Phoma solani* Cooke et Hark." (1892, p. 175); "*Phoma solanicola* Prillieux et Delacroix" (1892, p. 175).

first infection, dark streaks appearing along the petioles and spots upon the leaf surface. Within 12 days infection had become general, spots having been produced upon the youngest leaves as well as upon the oldest. The spots varied from pinpoint in size to areas 5 to 6 mm. in diameter. Frequently these spots united, forming irregular blotches, the tissue within which became dark-colored and shrunken, until finally the whole leaf yellowed and fell from the stem. Diseased leaves picked from plants under bell jars and from plants in the infection cages were brought to the laboratory for microscopical examination. Mycelium, pycnidia, and spores of the Phoma were found, and by means of agar plates the fungus was recovered in pure culture. The inoculated plants which were kept under bell jars for only 48 hours and then exposed to ordinary greenhouse conditions of temperature and moisture showed some spotting on the lower leaves. These spots, however, increased only slightly, and after three weeks the plants appeared to have thrown off the disease and were making good growth. A few of the leaves taken from a plant in the infection cage were photographed on March 20, 1914 (Pl. V). The average maximum temperature of air in the infection cage during 10 days (March 10–20, 1914) was 7.5° C. higher than that of the room, while the average minimum temperature of the cage was 4.1° C. lower than the room temperature.

POTATO TUBERS (*Solanum tuberosum*).—Potato tubers (Irish Cobbler variety) were thoroughly washed, soaked in a solution of mercuric chlorid (1 to 1,000) for two hours, and rinsed in distilled water. Stab inoculations were then made into the tubers from an oat-agar culture of the Phoma fungus, and the potatoes were placed in zinc boxes. The average temperature within the boxes was 25° C. during a period of four weeks, and the humidity was nearly 100 per cent, the boxes being kept wet with moist cotton and filter paper. At the end of eight days a slight darkening of the tissue was noticed about the needle stabs, but at the end of four weeks no definite spots had been produced. Four other inoculation experiments with potato tubers were tried, three in moist chambers at temperatures ranging from 14° to 29° C. with a low humidity and one at a temperature of 35° with high humidity. Under these conditions no infection occurred.

SUGAR-BEET PLANTS (*Beta vulgaris*).—Young sugar-beet plants growing in pots at the greenhouse were inoculated on January 28, 1914. The plants, which were 10 to 16 cm. high, were sprayed with a spore suspension of the Phoma fungus and placed under bell jars. Control plants were used. The plants were examined at intervals of several days, but showed no spotting in a period of three weeks.

Half-grown sugar beets growing in an open bed in the greenhouse were sprayed with a spore suspension of the fungus on February 4, 1914. These plants were not covered. In four weeks no spotting or other evidence of infection had taken place.

JIMSON WEED (*Datura tatula*).—On April 25, 1914, three mature plants of Jimson weed growing in pots at the greenhouse were sprayed with a spore suspension of the Phoma fungus. These plants were placed beneath bell jars for a period of 10 days, but no leaf spotting developed.

GARDEN PEAS (*Pisum sativum*) AND BEANS (*Phaseolus vulgaris*).— Young plants, 10 to 12 cm. high, growing in greenhouse pots, were inoculated on April 16, 1914, by spraying with a spore suspension of the Phoma fungus. The spraying was done with an atomizer on a cloudy day at a room temperature of 78° F. The plants were not covered. In two weeks no spotting had occurred upon either pea or bean leaves.

A second experiment with bean plants was tried similar to the preceding, except that the plants were placed beneath bell jars after the spraying. At the end of 10 days there was no infection.

PEPPER PLANTS (*Capsicum annuum*).—In February, 1914, some pepper plants just coming into blossom were sprayed with the Phoma spore suspension. The plants were kept under bell jars for 10 days. No infection could be seen after a period of three weeks. Other pepper plants were sprayed March 10, 1914, and placed in an infection cage. There was no infection in two weeks' time.

REISOLATION OF THE FUNGUS

FROM PLANTS INOCULATED IN GREENHOUSE.—Spots have been repeatedly produced by means of inoculations upon tomato fruit growing on greenhouse plants, and the Phoma fungus has been plated out from the discolored tissue. The following inoculations are typical of many others. In February, 1914, diseased spots were produced upon green tomato fruit by means of needle-prick inoculations. From tissue cut from the spots the fungus was recovered in agar plates. Diseased spots were produced upon mature fruit growing on greenhouse vines (Pl. III, fig. 2), and the fungous was isolated in pure cultures on February 2, 1914. The leaves of young and of mature tomato plants were diseased as a result of being sprayed with a fungous suspension in January, 1914. From the spotted leaf tissue taken from both young and old leaves the Phoma was isolated by means of agar plate cultures.

FROM PLANTS INOCULATED IN LABORATORY.—Green, partially ripened, and fully matured tomato fruits were picked from vines growing in the greenhouse and brought to the laboratory for inoculation. Upon these tomatoes spots were readily produced in four to seven days by means of needle-prick inoculations of the Phoma, and the fungus was recovered in pure cultures from tissue taken from the spots.

FROM PLANTS INOCULATED IN FIELD.—From tomato fruit, stem, and leaf tissue diseased through inoculation of the Phoma the fungus was obtained in pure culture by means of agar plates (Aug. 19, 1914).

DESCRIPTION OF THE FUNGUS ON ITS HOST

CHARACTER OF SPOTTING

The Phoma fungus produces spots on green and on ripe tomato fruit and on tomato leaves. The fruit, so far as observed, becomes infected only through wounds, while infection of the leaves and stems may be produced without wounding by means of spore spraying. The spots on ripe tomato fruit are black, carbonaceous, definite in outline, and have the surface covered with small black pycnidia. Surrounding the dark tissue of the spot is a watery-looking zone. Frequently the tissue breaks, and a grayish white mycelium develops in the cracks. The spots produced upon green fruit vary from brown to black in color and are membranous, with scattered pycnidia on the surface. Leaves of both young and mature tomato plants are susceptible to spotting. The spots are brown to almost black, definite in outline, and variable in size. Frequently several spots coalesce, forming irregular blotches. The lower leaves became infected first in the experiments, spots appearing upon blade and petiole. Within two weeks after inoculation by spraying, the leaves had become discolored and shriveled and were falling from the stem. Upon the surface of the leaf spots small dark pycnidia occurred singly or in groups. Reisolations in plate culture were made from tissue cut from diseased spots.

MYCELIAL GROWTH

The mycelium of this fungus forms a dense network of hyphal threads within the tissues of the host plant. The hyphæ are septate, frequently branching, and are hyaline to brown in color. Various stages in the development of pycnidia were observed in the tissue of the host, from the first intertwining of hyphal threads to the formation of a definite fruiting chamber, having dark irregularly shaped outer cells. Numerous oil drops were observed in hyphæ grown upon artificial media.

PYCNIDIAL DEVELOPMENT

The pycnidia are subglobose, membranous to carbonaceous, smooth, slightly papillate with a distinct central pore (occasionally two pores), color varying from almost hyaline to black, according to age and medium. There is considerable variation in size. Pycnidia occur scattered or in groups. They are at first covered by the epidermis, but later break through, the spores issuing in long coils through a central pore. In culture the flesh-colored spore exudate frequently forms a slime on the surface of the medium. The pycnidial wall is thin, with brownish outer cells and hyaline inner cells. One-celled pycnospores are developed from the end of the delicate filiform basidia which arise from the inner cells.

Pycnidia from cultural media and from tomato fruit were measured, with the following results:

Corn-meal culture. Average of 10 pycnidia, 151μ, variation 107 to 174μ.
Beef-agar culture. Average of 10 pycnidia, 103μ, variation 59 to 211μ.
Tomato fruit. Average of 40 pycnidia, 139μ, variation 53 to 348μ.

From these measurements it will be seen that an average of 60 measurements gives a diameter of 131μ, with a variation ranging from about 50 to 350μ.

PYCNOSPORES

Only one kind of spore is produced in the pycnidium, the hyaline 1-celled pycnospore. These spores are continuous, 2-guttulate, subcylindrical to subglobose in shape, with blunt, rarely tapering ends, and are produced singly on the unbranched filiform basidia which arise from the inner hyaline cells of the pycnidial wall.

No definite stroma is formed and no perithecial stage has been observed either in culture or upon the host tissue.

Pycnospores were found to vary considerably in size. The following measurements (Table IV) were made of spores grown upon media favorable to their development.

TABLE IV.—*Variation in size of pycnospores of the Phoma fungus*

Source of spores.	Number measured.	Average size.	Variation in size.
		μ	μ
Tomato fruit......................	40	4. 9 by 2. 2	3. 4 to 8. 5 by 1. 7 to 3. 7
Tomato leaf.....................	20	4. 6 by 2. 7	3. 2 to 6. 8 by 1. 7 to 3. 4
Potato cylinder..................	10	5. 7 by 2. 3	3. 4 to 8. 5 by 1. 7 to 3. 4
Melilotus stem.....................	10	4. 5 by 2. 2	3. 0 to 5. 4 by 1. 8 to 3. 2
String-bean agar..................	20	4. 7 by 2. 6	3. 4 to 6. 8 by 1. 7 to 3. 4
Corn-meal culture..............	40	4. 3 by 2. 1	3. 4 to 6. 8 by 1. 7 to 3. 5

The average size of 140 pycnospores is found from these measurements to be 4.7 by 2.3μ, with a variation of 3 to 8.5 by 1.7 to 3.7μ.

CULTURAL CHARACTERISTICS OF THE FUNGUS

The fungus grows well on all of the media commonly used in culture, good spore development being obtained on corn meal, string-bean agar, oat agar, potato cylinders, and tomato stems. Cultural characteristics of the fungus upon a number of different kinds of media are here given.

BEEF AGAR.—Fungous colonies appear on beef agar (+15) plates within two to three days. These colonies, varying in size from pin points to 5 mm. in diameter, are round, whitish, and somewhat cottony in appearance. The hyphæ branch freely, radiating toward the circumference of the colony. Beef agar is not favorable for spore development, although pycnidia and spores are sometimes produced.

IRISH-POTATO AGAR.—Whitish mycelial growth appears within two to three days. At first cottony in appearance, it later forms a compact mat of hyphal threads. In 5 to 7 days the medium darkens, and in 10 days pycnidia and spores have developed. Pycnidia develop more slowly and less abundantly upon this medium than upon the string-bean, prune, or oat agars.

STRING-BEAN AGAR.—Round whitish fungous colonies appear in two to three days. A few days later the surface growth becomes a compact mat, neutral gray in color, beneath which lies a darker substratum containing the pycnidia. Mycelial growth is more abundant than on beef agar, and the colonies are fuller, with somewhat convex surfaces. Pycnidia form within 6 to 10 days and within 10 to 14 days an exudate may be seen at the apex of the pycnidium, at first in the form of drops and later as a thick slime overspreading the surface of the culture.

LIMA-BEAN AGAR.—A whitish cottony growth appears within three to five days and a few days later pycnidia form, causing a darkening of the substratum. The pycnidia are round to irregular in outline, separate or in clusters, and show the characteristic slimy exudate.

PRUNE AGAR.—In two to three days there is a whitish fungous growth, which a few days later becomes grayish green in color. Single hyphæ when examined under the microscope appear olive green. These hyphæ contain numerous oil drops which disappear when treated with ether. With pycnidial development a dark, almost black color is produced in the substratum, while the spore exudate forms a slime on the surface.

OAT AGAR.—An abundant mycelial growth develops within three to five days. Later this growth darkens as a result of pycnidial development and there is an abundant spore production.

SYNTHETIC AGAR.[1]—A scant mycelial growth develops within three to five days, at first whitish but later becoming neutral gray in color. Spore development is poor.

STERILIZED POTATO CYLINDERS.—Within two to three days a whitish mycelium develops upon the surface of the potato plant. As growth continues, the mycelium becomes compact, varying in color from Ridgway's[2] neutral gray to mouse gray. With the development of pycnidia the substratum darkens and at the end of two weeks the growth becomes dark and feltlike, showing drops of exudate on the surface. Six-weeks-old potato cultures become carbonaceous in appearance and soot-black in color.

STERILIZED COTTON STEMS.—Upon this medium there is scant mycelial growth in four to five days. Later the stem becomes covered with a dark growth consisting of hyphal threads, dark pycnidia, and spore masses.

STERILIZED SWEET-CLOVER STEMS (*Melilotus alba*).—A scant grayish white mycelium develops upon the surface of the stem. Later the sub-

[1] Darwin, Francis, and Acton, E. H. Practical Physiology of Plants. 321 p., illus. Cambridge, England, 1894.

[2] Ridgway, Robert. Color Standards and Color Nomenclature . . . 43 p., 53 col. pl. Washington, D. C., 1912.

stratum darkens and the pycnidia empty their spore contents to the surface.

STERILIZED TOMATO STEMS.—Green tomato stems sterilized in 5 c. c. of distilled water afford a good medium for growth. A whitish cottony mycelium is first seen. This later develops into a dark compact matlike growth which covers the stem and spreads over the surface of the water as a firm pellicle. Within and upon the surface of this mat numerous dark pycnidia develop, and from these the spore masses issue in coils. Microscopical examination of 3-weeks-old tomato-stem cultures show well-developed hyphæ, mature pycnidia, and an abundance of spores. From glycerin slides obtained from these cultures drawings were made showing hyphæ, pycnidia, and spores (Pl. I, figs. 4 and 7). Spores from tomato-stem cultures varied in size from 3.4 to 5.1μ in length and from 1.7 to 2.5μ in width.

STEAMED CORN-MEAL CULTURES.—In sterilized corn-meal flasks (capacity 100 c. c.) growth begins to show in two or three days as a slightly darkened spot. In five days a scant radiating mycelium may be seen and in eight days there is a dark fungous growth 3 to 4 cm. in diameter. Pycnidia are thickly distributed throughout, from the mouths of which slimy spore masses issue in coils. Within two weeks after inoculation the entire surface of the corn meal becomes covered with a dark, compact, slightly greenish mat of fungus 1 to 2 mm. in thickness. The surface of this mat is pimply in appearance, owing to pycnidial development, and is often thrown into folds or depressions within which the exudate from the pycnidia accumulates (Pl. VI. fig. 2). The pycnidia are subglobose and vary in color from hyaline to dark brown and black. Single hyphal strands are brownish and contain numerous oil drops. In microtome sections made of mature pycnidia, fragile threadlike basidia were seen. Upon these basidia the hyaline 1-celled pycnospores are borne. These are subglobular to subcylindrical in shape, 1- to 2-guttulate, and vary considerably in size. In older corn-meal cultures (8 to 10 weeks old) the fungous growth becomes crustlike in composition and soot-black in color, breaking easily when touched with a needle. On the whole the best spore production (with scant mycelium) was obtained upon sterilized corn-meal and tomato-stem cultures. Good mycelial and spore development occurred on oat, string-bean, and lima-bean agar and upon potato cylinders.

Beef agar and synthetic agar proved very poor media for the growth of the fungus.

Characteristic growth upon Melilotus stem, string-bean agar, and potato cylinder is shown in Plate VI, figure 1.

VITALITY OF THE FUNGUS IN CULTURE

The vitality of the Phoma fungus in culture is good. Transfers made from potato cylinders and string-bean agar cultures 6 to 8 months old

showed growth in five days. Under ordinary laboratory conditions stem cultures (tomato, cotton, and sweet clover) dry out in less than a year and the fungus dies. Special medium was used in the hope of obtaining a perithecial stage of the fungus, but no fruiting bodies other than pycnidia have been observed in culture or upon host tissue.

TEMPERATURE RELATIONS

The Phoma fungus grows well at ordinary room temperatures. Transfers were made from a 2-weeks-old corn-meal culture to string-bean agar, potato cylinders, and corn-meal flasks and kept in incubators for a period of 10 days with the results given in Table V.

TABLE V.—*Temperature relations of the Phoma fungus*

Period of growth.	Average temperature (°C.).								
	0.7	1.3	4.9	5.6	7.9	10	14.4	15.6	23.7
10 days.... When removed to room temperature.	o Growth within 2 days.	o Growth within 2 days.	o Growth within 2 days.	o Growth within 2 days.	Trace. Continued growth.	Fair. Continued growth.	Good. Continued growth.	Good. Continued growth.	Good. Continued growth.

Period of growth.	Average temperature (°C.).								
	27.3	27.8	29.5	31.8	32.7	33.1	33.2	33.8	35.8
10 days.... When removed to room temperature.	Good. Continued growth.	Abundant. Continued growth.	Abundant. Continued growth.	Trace. Continued growth.	Trace. Continued growth.	o Growth within 5 days.	o Growth within 5 days.	o Growth within 5 days.	o o

These tests show the minimum temperature of the Phoma fungus to be about 6° C., the optimum temperature about 28°, and the maximum temperature between 32° and 33°.

DISTRIBUTION OF THE DISEASE

Information concerning the distribution of this Phoma disease of tomato has thus far been obtained only through specimens sent in for examination from Cutler, Fla., in 1912; Punta Gorda, Fla.; Cuba; Florence, S. C.; Miamisville, N. Y.; and Herrington, Kans., in 1914.

It seems probable that the disease is widely distributed but because of the presence of associated fungi has often been looked upon as one of the more commonly known fruit-spots. The seriousness of the disease under favorable climatic conditions is suggested in the report of Mr. James Brown, already mentioned, that this fruit-spotting had caused heavy loss among the farmers of Dade County, Fla., in 1912.

TAXONOMY OF THE FUNGUS

The earliest mention of a Phoma fungus as parasitic on tomato fruit is given by Plowright (1881). This fungus associated with three others is given as the cause of a "black spot" upon the crown of the fruit, and is described as follows:

Phoma destructiva. Perithecia carbonaceous, minute, globose, spherical clustered spores, hyaline, oval, cylindrical, binucleate, 5–6 mk. long, by 1.5–1 wide.

Spegazzini (1881, pp. 67–68) published a description of *Phyllosticta hortorum*, a fungus which he found parasitic on the leaves of the eggplant, and Ellis and Everhart (1900) state that it is common on tomato leaves. That there has been considerable confusion in regard to the identity of *Phyllosticta hortorum* (Speg.) is brought out in a recent article by Harter (1914), who sums up the situation in these words (p. 337):

If, therefore, any value is to be given to a comparison made by an author with his own type specimens, it is safe to conclude that *Phyllosticta hortorum* has not yet been found in this country.

In view of this statement, it is doubtful whether the eggplant fungus reported on tomato leaves was *Phyllosticta hortorum* (Speg.).

Cooke (1885, p. 94) thus describes *Phoma lycopersici* (*Phoma herbarum* West.):

Caulicolus. Perithecia punctiform, black, densely gregarious, at first covered by the cuticle, ultimately more or less exposed. Sporules lanceolate, binucleate (0.012 × 0.004 mm.). On stems of tomato.

No mention is made of the occurrence of this fungus upon the fruit, and the size of spores as given is about twice the average size of those of the Phoma described in this paper.

Peck (1887, p. 57) gives the following description of *Phyllosticta lycopersici* as a parasite upon tomato fruit:

Spots large, suborbicular, cinereous; perithecia minute, brown or blackish, opening by a single or sometimes by two pores; spores abundant, oblong or elliptical, .00025 to .0003 inch long, .0001 to .00012 broad. Fruit of tomato.

Except for slight differences in size and shape of spores, this description is similar to that given by Plowright for *Phoma destructiva*.

Marchal (1900) mentions a Phoma as the cause of a new disease of greenhouse-grown tomatoes, but gives no specific name to his fungus.

In order to determine if possible something more definite in regard to the relation between *Phoma destructiva* Plowr. and *Phyllosticta lycopersici* Pk., a type specimen of Peck's fungus was requested and kindly furnished by him from the herbarium of the New York State Museum in October, 1914. This specimen (dated July, 1886) consisted of a small piece of tomato fruit showing a portion of a diseased spot, the tissue of which varied from Ridgway's buff [1] to olive brown in color. On account of the age of the specimen and the presence of a foreign fungus in the tissue,

[1] Ridgway, Robert. Op. cit.

identification of the causal organism was difficult, but in slides made from different parts of the discolored tissue some brownish subglobose pycnidia and a few hyaline 1-celled spores of *Phyllosticta lycopersici* were observed. Efforts made to secure cultures of Phyllosticta from the type specimen were unsuccessful. From descriptions of the fungi given by Plowright and Peck and from examination of the type specimen just described in the opinion of the writer it appears that *Phoma destructiva* Plowr. and *Phyllosticta lycopersici* Pk. are synonymous and that the Phoma described in this article is identical with them. Because of these facts and in order to avoid confusion of names, it has been thought best to adopt Plowright's nomenclature. The fungus described in this paper as causing fruit-rot, stem- and leaf-blight of the tomato is therefore designated as *Phoma destructiva* Plowr. emend.

Phoma destructiva Plowr. emend.

> *Phoma destructiva* Plowr., 1881, *in* Gard. Chron., n. s. v. 16, no. 411, p. 620.
> *Phyllosticta lycopersici* Pk., 1887, *in* 40th Ann. Rpt. N. Y. State Mus. Nat. Hist., 1886, p. 57.
> *Phoma* sp. Marchal, 1900, *in* Bul. Agr. [Brussels], t. 16, no. 1, p. 18.

Diagnosis.—Spots on tomato fruit, brown to black, membranous or carbonaceous, definite. Pycnidia scattered to aggregate, most abundant toward center of spot, subcutaneous later erumpent, glabrous, brownish black, subglobose, slightly papillate, not beaked, ostiolate (usually one, sometimes two pores), 30 to 350μ in diameter; pycnidial wall thin, outer cells brown, inner cells hyaline; delicate filiform basidia arising from inner cells. Pycnospores issue in coils through ostiolum, forming a slimy flesh-colored exudate; hyaline, continuous, 1-celled, 2-guttulate, subcylindrical to subglobose, rarely tapering, variation (100 measurements) 2.8 to 8.5 by 1.7 to 3.4μ; produced singly on unbranched filiform basidia. Hyphæ septate, branching, hyaline to brownish, vacuolate. No definite stroma. No perithecial stage observed.

Habitat.—Parasitic on fruit of *Lycopersicon esculentum*, spots occurring on tomatoes (green and ripe), usually near stem end, 1 to 3 cm. Conspicuous, dark, definite, sometimes coalescing; tissue membranous to carbonaceous, depressed, with dark, minute, pimple-like pycnidia. Observed upon tomato fruit received from Florida, South Carolina, Kansas, New York, and Cuba.

CONCLUSIONS

As a result of inoculation experiments, *Phoma destructiva* Plowr. emend. has been proved an active wound parasite upon green and ripe tomato fruit.

This fungus also causes a leaf spotting of tomato and potato plants.

LITERATURE CITED

COOKE, M. C.
> 1885. New British fungi. *In* Grevillea, v. 13, no. 68, p. 89–100.

ELLIS, J. B., and EVERHART, B. M.
> 1900. North American Phyllostictas . . . 74 p. Vineland, N. J.

HARTER, L. L.
> 1914. Fruit-rot, leaf-spot, and stem-blight of the eggplant caused by Phomopsis vexans. *In* Jour. Agr. Research, v. 2, no. 5, p. 331–338, pl. 26–30.

MARCHAL, Émile.
> 1900. Rapport sur les maladies cryptogamiques . . . *In* Bul. Agr. [Brussels], t. 16, no. 1, p. 9–21.

Peck, C. H.
  1887. Report of the botanist. *In* 40th Ann. Rpt. N. Y. State Mus. Nat. Hist.,
    1886, p. 39-77.
Plowright, C. B.
  1881. On the fungoid diseases of the tomato. *In* Gard. Chron., n. s. v. 16, no. 411,
    p. 620-622, fig. 117-124.
Saccardo, P. A.
  1884. Sylloge Fungorum . . . v. 3. Patavii.
  1892. Sylloge Fungorum . . . v. 10. Patavii.
Spegazzini, Carlos.
  1881. Nova addenda ad mycologiam venetam. *In* Atti Soc. Crit. Ital., ann. 24,
    s. 2, v. 3, disp. 1, p. 42-71.

PLATE A

Tomato fruit spotted with *Phoma destructiva*.

Fig. 1.—Natural infection on tomato received from Cutler, Fla., March, 1912.

Figs. 2, 3, 4, and 5.—Spots produced as the result of needle-prick inoculations with *Phoma destructiva*.

Painted by J. Marion Shull.

PLATE B

Potato and tomato leaflets spotted as result of inoculation with *Phoma destructiva*.

Fig. 1.—Potato leaflet from sprayed plant.
Figs. 2 and 3.—Tomato leaflets from sprayed plant.
Painted by J. Marion Shull.

PLATE I

Fig. 1. *Phoma destructiva:* Group of pycnidia, with surrounding mycelium, show-
ing relation of the parasite to the host tissue. *P*, Pycnidia; *M*, mycelium; *C*, cell of
host. Drawn by J. F. Brewer.

Fig. 2.—*Phoma destructiva:* Single hyphæ, showing septation and branching.
Drawn by J. F. Brewer.

Fig. 3.—*Phoma destructiva:* A few pycnospores highly magnified. Drawn by J. F.
Brewer.

Fig. 4.—*Phoma destructiva:* Pycnidium and surrounding mycelium. Side view,
showing spores issuing from ostiolum. Drawn by J. F. Brewer.

Fig. 5.—*Phoma destructiva:* Pycnidium as seen in cross section of diseased tissue of
tomato fruit. Mass of pycnospores are within pycnidial chamber. Surrounding the
pycnidium are cells of host tissue and cut hyphal threads. Drawn by J. F. Brewer.

Fig. 6.—*Phoma destructiva:* Pycnidium from artificial culture. Cross section show-
ing cell structure of wall, basidia, and pycnospores. Semidiagrammatic drawing by
J. F. Brewer.

Fig. 7.—*Phoma destructiva:* Pycnidium and surrounding mycelium. View from
above, showing ostiolum. Drawn by J. F. Brewer.
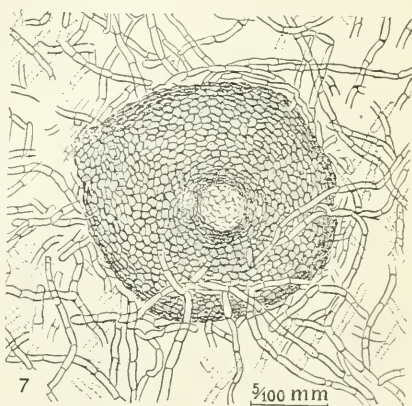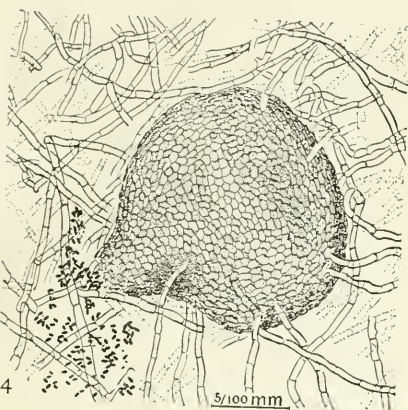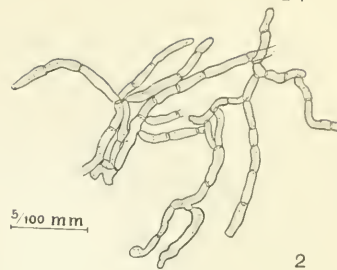
PLATE I

PLATE II
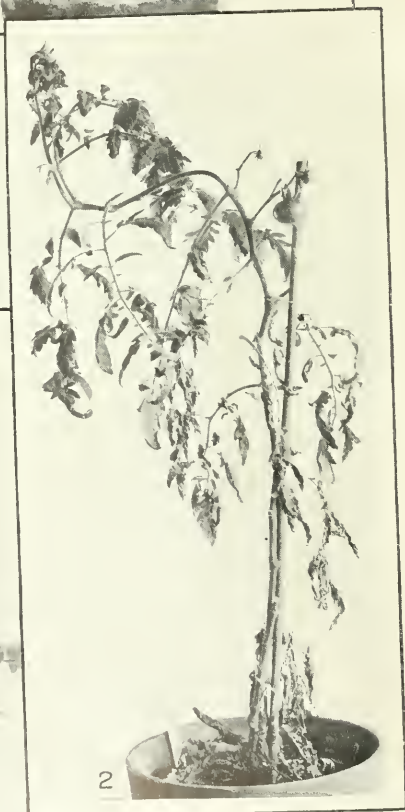
Phoma Destructiva

PLATE II

Mature and young tomato plants grown in greenhouse, showing infection by *Phoma destructiva*.

Figs. 1 and 2.—Mature plants. Fig. 1.—Control plant. Fig. 2.—Diseased plant, 10 days after spraying with a spore suspension of *Phoma destructiva*. Foliage spotted and lower leaves fallen from stem.

Figs. 3 and 4.—Young plants with foliage spotted as a result of inoculation with *Phoma destructiva*. Photographed eight days after inoculation.

1

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PLATE IV

PLATE IV

Tomato leaves showing spots produced by spraying with a suspension of *Phoma destructiva*. Photographed 12 days after treatment.
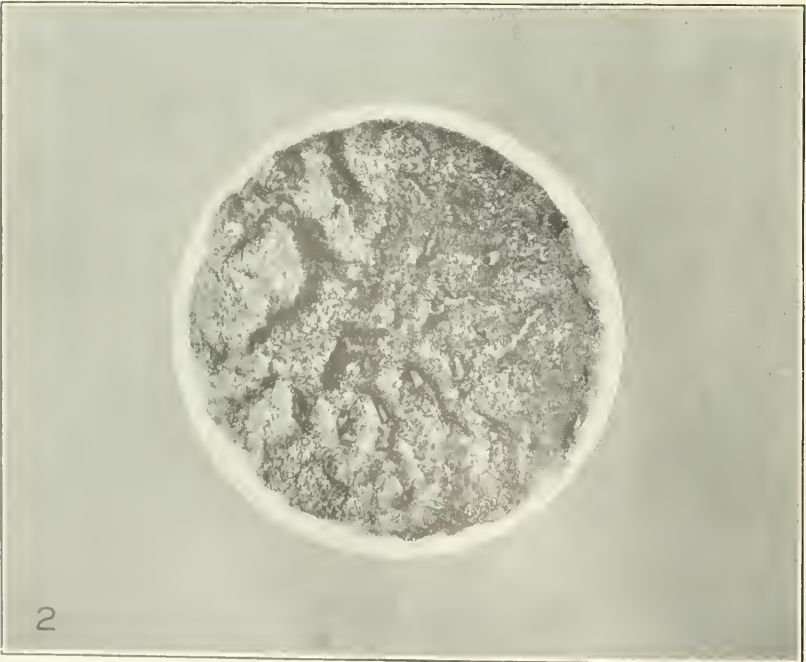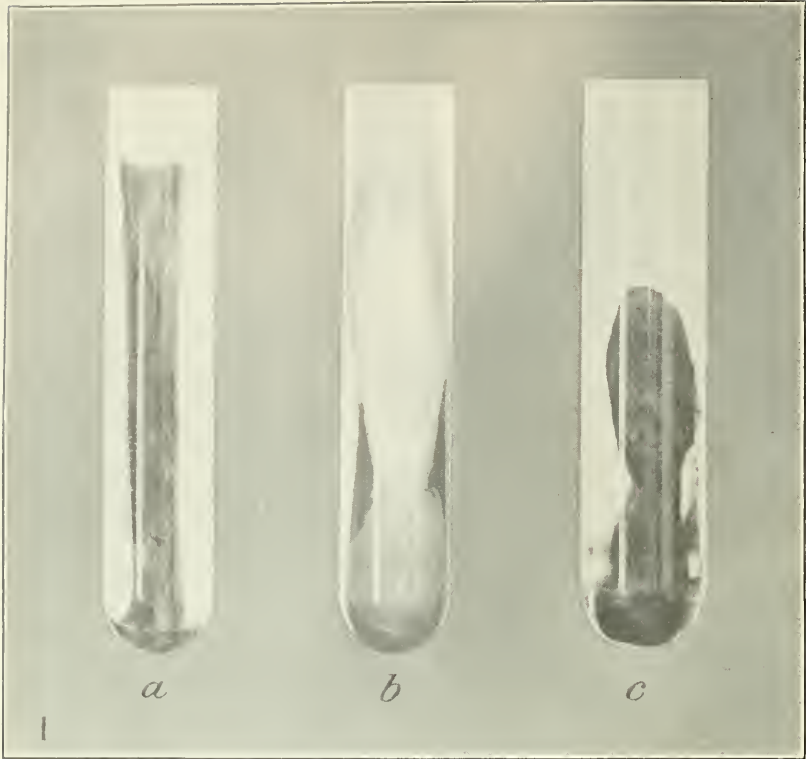
PLATE VI

PLATE VI

Phoma destructiva: Artificial cultures.

Fig. 1.—Test tube cultures. *a*, Melilotus stem; *b*, string-bean agar; *c*, potato slant.
Fig. 2.—Corn-meal flask culture.

AVAILABILITY OF THE NITROGEN IN PACIFIC COAST KELPS

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PURPOSE OF THE STUDY

One of the first problems to be solved in the study of the fertilizing value of the three major kelps of the Pacific coast, *Macrocystis pyrifera*, *Nereocystis luetkeana*, and *Pelagophycus porra*, is the form in which these plants can most economically and completely be utilized. The difficulties inherent in any plan for manufacturing pure potash salts as their sole product have already been pointed out by J. S. Burd (5),[1] of the California Agricultural Experiment Station. It has also been shown in the same publication that the most feasible plan of manufacture so far suggested is to dry and grind the kelp and to use it either alone or preferably in combination with other ingredients as the basis for a mixed fertilizer. The advantages of such a method of operation are at once obvious. The low cost of operation and the probable complete utilization of the plant food present are among the most evident. In the latter connection, however, it at once becomes very pertinent to inquire what will be the probable fate of the organic matter of the kelp when added to the soil.

Will it readily decompose and aid in the formation of humus? Will its nitrogen and phosphoric acid become freely available for the crops upon which it is used?

In addition, it will be very desirable to know what effect not only the organic matter of the kelp but also its salts will have upon the biological, chemical, and physical processes of the soil and also upon the plants growing therein. The laboratory studies taken up in this paper are intended to show the availability of the organic matter of the kelps studied and also to give some insight into the effect of the salts present in them upon the biological activities of the soil.

The study of the changes taking place in organic matter when added to the soil is of the most absorbing interest to modern investigators. It has been the subject of many excellent researches during recent years, though earlier work dates back to the time of Boussingault (2) in 1834. At present the most satisfactory procedure for studying this problem is undoubtedly the so-called beaker method of soil bacteriology, which measures the availability of a material by the readiness with which it will ammonify and nitrify when a small portion is added to a definite quantity of soil. This method is now so well known that it hardly

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[1] Reference is made by number to "Literature cited," pp. 37–38.

needs further description, and the reliability of the results obtained by it has been fully demonstrated through the work of a large number of investigators who have taken it up since it was first proposed by Stevens and Withers (18, 19, 20). Lipman and Brown (11), working together at the New Jersey Experiment Station, have proposed the same manner of experimentation, and this method of investigation has been almost universally adopted. The same general procedure was therefore considered to be suitable for the study of the availability of the organic matter of the kelps in question.

EXPERIMENTAL METHODS

In order that the results should be comparable to those obtained under field conditions, the soils used throughout all the series were fresh field soils. The surface soil was removed to a depth of $1\frac{1}{2}$ to 2 inches, it having been shown by Löhnis (15, p. 571) and others that this portion does not possess a typical bacterial flora. A large sample was then taken from the next 3 or 4 inches of soil, carried immediately to the laboratory, spread out in a darkened room, and dried to a point where it could be readily sifted and thoroughly mixed. In this condition it was still moist and, according to the work of Brown (3, 4), undoubtedly possessed a bacterial flora which was representative of field conditions. Two-hundred-gram portions of this soil were then placed in sterile tumblers, which were covered with Petri dishes. The various amounts of material to be investigated were then added, the whole being thoroughly mixed. Sterile distilled water was added to make optimum moisture conditions, which in most cases were found to be about 18 per cent. The samples and duplicate blanks were then incubated at 28° to 30° C. for the required time. At the end of the period they were transferred to copper distilling flasks. About 400 c. c. of distilled water were added, together with magnesium oxid and a small quantity of paraffin to prevent foaming. The ammonia present was distilled into standard $N/10$ hydrochloric acid and the excess portion titrated with standard $N/10$ ammonia in the usual manner.

Upon consideration of the analytical results obtained from the first portion of Series I and the very slow rate of ammonification of *Macrocystis pyrifera* revealed by these results, it seemed desirable to have determinations of the amount of nitrification which was probably taking place concurrently. This would, it was felt, be a satisfactory check on the progress of the transformation of the nitrogen through the cycle of changes taking place in the soil. The residues remaining in the copper distilling flasks after the determination of ammonia were immediately transferred to 1-liter volumetric flasks of the Giles pattern, or, where much small gravel was present, to 1-liter graduated cylinders. They were then made up to 1 liter and sufficient additional distilled water added to compensate for the displacement caused by the soil and ferti-

lizing material present, this figure being determined for each set on other duplicate portions. The whole solution was thoroughly mixed and an aliquot portion taken for the determination of nitrates by the aluminum-reduction method. The essential procedure as outlined by the American Public Health. Association (1, p. 25) was followed. Previous work with this method on sewage samples, which were solutions of essentially the same type, had shown it to be very satisfactory for nitrate determinations. Burgess (6) has also found this method excellently adapted to the determination of nitrates in soils. The accuracy of the above procedure was also checked by running several series of triplicate determinations in which 200 gm. of soil were taken and distilled with magnesium oxid, as shown above, and the nitrates in the residue determined. To other triplicate sets varying quantities of potassium nitrate were added. They were then similarly analyzed. The agreement throughout was excellent. In some cases it was absolute. In none did the divergence exceed the ordinary analytical variation.

SERIES I.—COMPARATIVE AMMONIFICATION AND NITRIFICATION OF DRIED KELP, DRIED BLOOD, AND COTTONSEED MEAL, USING CLAY ADOBE SOIL

In the first series *Macrocystis pyrifera*, *Nereocystis luetkeana*, and *Pelagophycus porra* were each used in the proportion of 10 gm. of dried and ground kelp to 200 gm. of fresh field soil. This soil was taken from the campus botanical gardens and was in texture a slightly modified clay adobe. In order to compare the kelp with substances of well-known availability, portions of 1 gm. of dried blood and 2 gm. of cottonseed meal were also added to duplicate sets of tumblers, while blank determinations of untreated soil were started to determine the ammonia and nitrate production occurring in the natural soil. The kelp employed was in each case a composite made up from the analytical samples of that variety. These had been dried to a constant weight at 100° to 105° C. for a period of three to nine hours and then ground so as to pass through a 0.5-mm. sieve. The percentage composition of the kelp was as follows:

Species of kelp.	Moisture.	Nitrogen.	Total soluble salts.
Macrocystis pyrifera.	4. 71	1. 07	30. 77
Nereocystis luetkeana.	5. 24	1. 66	46. 83
Pelagophycus porra.	4. 36	1. 22	45. 64

It will be noted that the moisture content was fairly uniform in all varieties, while both the *N. luetkeana* and *P. porra*, as usual, contained larger quantities of nitrogen and total soluble salts than the *M. pyrifera*. The composition of the salts, both soluble and insoluble, added to the soil when applying kelp will be of considerable interest in this connection. It was impossible to make complete ash analyses of all the kelps used throughout these studies. Mr. P. L. Hibbard, of this laboratory, however, has made analyses of representative plants of each species, and it is

felt that these will be fairly typical of the materials used in this investigation. The figures as quoted are expressed as the percentages of actual radicles occurring in the ash, and the total percentage of ash is the amount found in the plants when calculated to a moisture-free basis.

Composition of the ash of the harvestable portions of Macrocystis pyrifera, Nereocystis luetkeana, and Pelagophycus porra

Constituent.	Macrocystis pyrifera.	Nereocystis luetkeana.	Pelagophycus porra.
	Per cent.	Per cent.	Per cent.
Ca.	4. 96	2. 10	2. 09
Mg.	2. 24	1. 55	1. 71
Na.	10. 52	11. 05	8. 63
K.	29. 46	32. 66	34. 73
Fe$_2$O$_3$.	} .43	.17	.26
Al$_2$O$_3$.			
Cl.	34. 93	40. 89	40. 83
SO$_4$.	7. 92	4. 63	4. 84
CO$_3$.	4. 44	3. 10	1. 66
PO$_4$.	2. 30	1. 91	2. 18
Total	97. 20	98. 06	96. 93
Total percentage of ash in water-free material.	35. 62	50. 57	52. 66

There are several very important deductions to be made from these analyses when considering them from the point of view of soil fertility. The very large percentage of total ash present is at once apparent; and from preceding determinations of the soluble salts present in similar samples it is evident that the larger portion of the total ash, about 85 per cent, is, in fact, water-soluble.

Some quantitative separations of the soluble salts were made which showed that small quantities of the calcium and traces of the magnesium only were dissolved, while all the potassium and chlorin and the major portion of the sodium and sulphate ions went into solution.

The immediate effect, then, of the incorporation of kelp in the soil is a very considerable addition of soluble salts. Three-fourths or more of these will be potassium chlorid or sulphate, while the other fourth will largely consist of sodium chlorid and sulphate. The potash will be fixed in the soil by its well-known precipitative power for this compound, while the sodium salts will in any ordinarily well-drained soil be carried off in the drainage water. As the kelp gradually decomposes, the compounds present in the soil will approach those represented in the complete ash analyses above given. They will then tend to make a much more balanced solution, even if all the sodium salts be not removed by that time.

From C. B. Lipman's work on the toxicity and antagonism of various salts on soil bacteria both in solutions and in soil (7, 8, 9, 10, 12) it would be reasonable to expect that if any disturbance of the biological activities of the soil is caused by the soluble salts from applications of kelp this influence would be very likely to decrease as the soil solution

becomes more balanced in its character. He has shown that the principles of antagonism previously established by Loeb (13, 14) and Osterhout (16, 17) for animals and plants also hold in general for soil bacteria.

Considering now the results obtained in Series I, it will be observed that the first ammonia determinations were made at the end of 9 days' incubation. Other duplicate sets were analyzed at periods of 11, 15, 19, 48, and 102 days. Even at the end of 9 days it will be seen that very striking differences exist. As was to be expected, the dried blood ammonifies most readily. Next in rate of conversion is the cottonseed meal, while *Nereocystis luetkeana* is not very far behind this. *Pelagophycus porra* is distinctly slower, while *Macrocystis pyrifera* shows only a trace converted.

Still considering the ammonification, the same relationship holds with the kelps throughout the whole series. After 15 days the major portion of the nitrogen, which was changed to ammonia with the blood and the cottonseed meal, has been converted over into nitrates. It must be pointed out in the discussion of the results of this first series that the amounts of kelp added were quite excessive. The only reason for employing such quantities even in a laboratory study was that it furnished an amount of nitrogen which would give a very satisfactory analytical figure should conversion readily take place. It had been supposed also that all the kelps were probably very available and would decompose readily, so the large quantities used were intended to test this belief under extreme conditions.

Taking this series as a whole, it will be seen that even with the large content of soluble salts furnished by the kelp, ammonification took place with surprising readiness in the case of Nereocystis and Pelagophycus, while with Macrocystis it was only after 48 days had elapsed that definite small amounts were converted to ammonia. Nitrification, however, in the case of all the kelps was almost entirely inhibited; in fact, even the amounts originally present in the soil did not remain at the end of the period in the form of nitrates. At the close of the final period, 102 days, duplicate sets of tumblers were mixed and the total amount of nitrogen determined by the modified Kjeldahl method to include the nitrogen of nitrates.

Material.	Recovered. Mg.	Added. Mg.
Macrocystis pyrifera	$\begin{cases} 100.\ 5 \\ 100.\ 5 \end{cases}$	107
Nereocystis luetkeana	$\begin{cases} 165.\ 7 \\ 162.\ 3 \end{cases}$	166
Pelagophycus porra	$\begin{cases} 114.\ 5 \\ 125.\ 9 \end{cases}$	122
Dried blood	$\begin{cases} 100.\ 8 \\ 92.\ 2 \end{cases}$	132
Cottonseed meal	124. 7	128

There has apparently been a slight denitrification in the case of the dried blood, but with the other materials the amount recovered is substantially the same as that which had been added.

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TABLE I.—Series I. Comparative ammonification and nitrification of dried kelp, dried blood, and cottonseed meal

[Soil used. Clay adobe from the campus botanical gardens, Berkeley, Cal.]

Material added to 200 gm. of soil	Quantity of material added (Gm.)	Quantity of nitrogen added (Mg.)	Quantity of soluble salts in kelp added (Gm.)	Incubated 9 days — Quantity of nitrogen gained in ammonia (Mg.)	Incubated 11 days — ammonia (Mg.)	Incubated 11 days — nitrates (Mg.)	Incubated 11 days — Average percentage of added nitrogen gained in ammonia and nitrates	Incubated 15 days — ammonia (Mg.)	Incubated 15 days — nitrates (Mg.)	Incubated 15 days — avg. %	Incubated 19 days — ammonia (Mg.)	Incubated 19 days — nitrates (Mg.)	Incubated 19 days — avg. %	Incubated 48 days — ammonia (Mg.)	Incubated 48 days — nitrates (Mg.)	Incubated 48 days — avg. %	Incubated 60 days — ammonia (Mg.)	Incubated 60 days — nitrates (Mg.)	Incubated 60 days — avg. %	Incubated 102 days — ammonia (Mg.)	Incubated 102 days — nitrates (Mg.)	Incubated 102 days — avg. %
Macrocystis pyrifera	10	107	3.08	0.34; .55	−0.07; .49	−0.95; .95	None	−0.35; .29	−1.57; −1.52	None	0.98; .35	−1.50; 1.50	None	1.96; 2.66	−4.24; −4.59	None	2.31; 4.56	−4.64; −4.64	None	2.87; 2.45	7.35; 7.30	None
Nereocystis luetkeana	10	166	4.68	23.30; 19.25	27.61; 33.81	1.75; .90	18.75	46.30; 30.90	.18; −1.32	22.91	47.83; 36.33	.20; .45	28.55	51.76; 62.76	−4.80; −4.86	31.59	61.29; 61.17	−3.20; −3.08	34.99	62.28; 60.32	5.43; 4.55	33.92
Pelagophycus porra	10	122	4.56	9.47; 6.95	9.47; 2.59	.95; .85	6.90	14.32; 15.44	−1.37; −1.37	11.07	16.47; 15.61	−1.20; −1.30	12.11	23.26; 18.22	−4.49; −4.49	13.31	21.37; 9.32	−4.20; —	14.34	22.91; 27.67	Lost; 6.55	18.74
Dried blood	1	132		44.32; 44.88	36.71; 39.71	4.75; 4.75	32.54	32.20; 33.28	46.33; 23.33	51.19	26.03; 33.61	64.80; 30.20	54.77	11.49; 11.63	65.20; 75.20	61.94	11.24; 3.01	75.00; 71.00	63.32	13.19; 16.16	53.25; 52.25	46.77
Cottonseed meal	2	128		30.28; 31.12	27.31; 26.61	2.75; 3.75	23.56	20.36; 18.82	13.33; 18.33	27.69	16.74; 15.46	30.20; 26.20	34.62	1.62; 4.35	71.20; 65.20	55.69	3.61; —	53.00; 55.00	44.54	3.85; 4.22	50.25; 60.25	46.34

SERIES II.—COMPARATIVE AMMONIFICATION AND NITRIFICATION ON FOUR TYPICAL SOILS OF FRESH AND DRIED MACROCYSTIS, COTTON-SEED MEAL, AND DRIED BLOOD

The results obtained in Series I clearly show the readiness with which *Nereocystis luetkeana* and *Pelagophycus porra* are decomposed in the soil with the formation of ammonia. With smaller concentrations of kelp, this would probably be readily converted over to nitrates. These varieties, however, are only of minor importance from a commercial standpoint. As has been pointed out by J. S. Burd (5), the Macrocystis is the only one which can be considered commercially important in California.

A second series of ammonification and nitrification studies was therefore started to further investigate the availability of *Macrocystis pyrifera*. It was felt also that the composite analytical sample of Macrocystis which was used in the first series had possibly been dried for a longer time than would be the case if the kelp were handled commercially.

In the second series, therefore, *M. pyrifera*, which, after having been thoroughly sun-dried, contained 10.52 per cent of moisture, 0.80 per cent of nitrogen, and 30.80 per cent of soluble salts, was employed. Fresh kelp, just gathered from the ocean, was also obtained at Pacific Grove, Cal., brought to Berkeley the same day, and placed in a refrigerator till it was used the following morning. The fresh kelp contained 87.9 per cent of moisture, 0.22 per cent of nitrogen, and 4.60 per cent of soluble salts. These two forms of kelp were again contrasted with dried blood and cottonseed meal. In order to study the effect on a variety of soils, four different types of fresh field soil were employed. The first was again the clay adobe from the university campus. The next was a highly productive alluvial loam from Hayward, Cal. A moderately productive clay loam was also obtained from Hayward, and for the fourth a light sandy soil from the vegetable-garden district of South San Francisco.

The series was divided into two sets. In the first set both the fresh and the dry Macrocystis were partially leached with distilled water. Any such treatment of the fresh kelp would be impracticable on a large scale. The thick colloidal solutions formed are extremely difficult to pour or filter. A great deal of moisture was also retained by the kelp. After leaching, the fresh sample contained 95.46 per cent of moisture, 0.09 per cent of nitrogen, and 1.46 per cent of soluble salts. The dried and ground Macrocystis was easily treated with about 15 c. c. of distilled water. This removed a few milligrams of nitrogen and two-thirds of the soluble salts. The tumblers of this set were incubated for 7 days.

In the other set the kelp was added to the soil without leaching, and the period of incubation was 11 days. The fresh kelp used in both sets was ground by passing it through a food chopper. The dry Macrocystis was ground, as in Series I, and the same analytical procedure was followed. (See Table II.)

TABLE II.—*Series II. Comparative ammonification and nitrification on four typical soils of fresh and of dried Macrocystis pyrifera, leached with distilled water and unleached, as contrasted with cottonseed meal and dried blood*

Period of incubation and material added to 200 gm. of soil.	Quantity of material added. (Gm.)	Quantity of nitrogen added. (Mg.)	Quantity of soluble salts in kelp added. (Gm.)	Clay adobe soil, Berkeley — ammonia (Mg.)	— nitrates (Mg.)	— avg. %	Highly productive loam from Hayward — ammonia (Mg.)	— nitrates (Mg.)	— avg. %	Moderately productive loam from Hayward — ammonia (Mg.)	— nitrates (Mg.)	— avg. %	Sandy soil from San Francisco — ammonia (Mg.)	— nitrates (Mg.)	— avg. %
INCUBATED 7 DAYS:															
Fresh *Macrocystis pyrifera*, leached	35	33.00	0.50	2.45	1.30	11.36	0.49	2.00	None.	0.21	4.40	9.76	2.03	0.65	9.06
				2.45	Lost.		.21	.00		.63	1.20		2.45	.85	
Dry *Macrocystis pyrifera*, leached	5	37.36	.51	.91	.50	.72	.49	3.00	None.	.35	1.86	None.	.07	.45	None.
				.63	Lost.		Lost.	3.00		.21	1.86		.21	.47	
Dried blood	1	132.00		52.75	3.80	41.85	13.01	8.80	19.48	47.84	2.50	37.75	56.25	2.25	44.34
				50.65	3.30		11.84	17.80		45.32	4.00		56.11	2.45	
Cottonseed meal	2	128.00		40.28	1.30	32.24	6.94	13.80	15.98	42.39	1.30	32.61	44.48	.25	34.37
				39.86	1.10		7.36	12.80		42.39	.00		43.50	.25	
INCUBATED 11 DAYS:															
Fresh *Macrocystis pyrifera*, unleached	35	77.00	1.63	9.04	1.00	12.44	.98	3.20	4.94	13.29	5.96	25.44	15.12	.65	17.61
				8.62	1.00		.42	3.20		14.41	6.56		14.01	.65	
Dry *Macrocystis pyrifera*, unleached	5	40.00	1.54	1.33	.70	None.	.56	4.60	None.	.17	2.88	None.	.14	.55	None.
				1.47	.66		.56	4.60		.17	2.84		.00	.75	
INCUBATED 9 DAYS:															
Dried blood	1	132.00		46.59	15.00	46.30	2.10	57.20	43.95	45.64	16.96	48.13	57.72	12.05	50.10
				46.45	14.20		1.54	55.20		45.50	18.96		56.46	6.05	
Cottonseed meal	2	128.00		36.08	8.60	35.00	.28	40.20	31.95	39.90	12.96	42.86	44.97	2.55	37.71
				36.92	8.00		1.12	40.20		39.90	16.96		44.97	4.05	

The analytical tables furnish some interesting results. It is at once evident that the fresh Macrocystis, whether leached or unleached, is readily decomposed by practically all of the four soils. It is also apparent that the leaching, which has removed a considerable portion of the soluble salts present, has not increased the rate at which the nitrogen is changed to more available forms. It was originally planned to incubate all of this series for 7 days. Upon analyzing the cultures with leached kelp, however, it was found that the decomposition had not been so rapid as anticipated, and it was supposed that the unleached samples would be slower still. They were therefore incubated for 11 days. The result proves that decomposition had been going on in this portion of the set as rapidly as in the leached. The removal of the salts from Macrocystis would not, therefore, be advantageous to increase its rate of decomposition. It is also interesting to note that nitrification has taken place with the fresh kelp in all of the soils except the sandy, a type which is frequently somewhat slow in this respect.

With the dry Macrocystis in this series both the leached and unleached gave practically a negative result. It was to be expected that there would be some disparity in the availability of the fresh and dried material. A green-manuring crop which is dried before it is turned under the soil differs somewhat similarly from the same crop turned under in a green condition, the fresh legumes decomposing more readily than the dried. The difference between the fresh and the dry Macrocystis is, however, very great. We have no proof as yet of the final availability of the dry Macrocystis. To demonstrate this, we require a positive gain of both ammonia and nitrates. It is of decided importance in our study to have found that the fresh Macrocystis will decompose with any average soil.

SERIES III.—THE EFFECT OF LARGE AND SMALL AMOUNTS OF DRIED MACROCYSTIS AND NEREOCYSTIS ON THE AMMONIFICATION AND NITRIFICATION OF DRIED BLOOD

The results so far obtained have left several points undecided. It has been indicated that oven-dry Macrocystis decomposes quite slowly, while Nereocystis at least ammonifies readily. Fresh Macrocystis both ammonifies and nitrifies. It now becomes desirable to know how long a period will elapse before dry Macrocystis in small or large quantities will decompose. Will dry Nereocystis in moderate amounts nitrify, as well as ammonify? And as kelp will probably be used with other organic fertilizers, how will materials like blood or tankage behave when the salts of kelp are also present?

The following series was therefore planned: Air-dried *Macrocystis pyrifera* and oven-dried *Nereocystis luetkeana* were each added to duplicate sets of tumblers. The amounts used furnished 10, 50, and 100 mg. of nitrogen. To another set of tumblers with the same three quantities of kelp, dried blood to furnish 10 mg. of nitrogen was added. In like

manner dried blood to furnish 100 mg. of nitrogen was added to other duplicate sets of kelp and soil. The above combinations were contrasted with duplicates containing 10 mg. and 100 mg. of nitrogen from dried blood, and also with tumblers of untreated soil.

The Macrocystis used contained 8.47 per cent of moisture, 0.90 per cent of nitrogen, and 35.68 per cent of soluble salts. The composition of the Nereocystis was 3.51 per cent of moisture, 1.70 per cent of nitrogen, and 46.47 per cent of soluble salts. The dried blood was the same as in Series I and II. The fresh field soil employed was the clay adobe from the campus botanical gardens. The ability to ammonify and nitrify which was shown by this soil in Series II was considered thoroughly satisfactory. It could be obtained readily in a fresh condition and for these reasons was used in this and all further work.

Several duplicate sets of tumblers with additions of Macrocystis and blood were prepared. These were incubated for periods of 2, 4, 11, and 15 weeks. Two sets of Nereocystis were incubated for 2- and 4-week periods, respectively. (See Tables III and IV.)

In Tables III and IV the total number of milligrams of nitrogen found as ammonia and as nitrates has been given, as well as the number of milligrams gained. This has been done because these data in some cases give more information than those recorded under the head of gain.

The results where the Macrocystis is the only addition to the soil will first be considered. The tumblers with 10 mg. of added nitrogen from kelp have apparently too small an amount present to yield any definite result. The variations in each period between the treated tumblers and the untreated soil are insignificant. The addition of 50 mg. of nitrogen in Macrocystis produces some striking changes. At the end of 2 and 4 weeks the result is the same; a small gain of ammonia has taken place. This is accompanied by a great reduction in the quantity of nitrates present. At 11 weeks there is not only an appreciable gain of ammonia, but the nitrate production is almost normal. At the end of 15 weeks the ammonia present is the same as in the blank, and 6 per cent of nitrogen has been gained in nitrates. This is considered to be an appreciable figure, especially in view of the improved nitrate content shown in the previous set. The results from the tumblers with 100 mg. of added Macrocystis nitrogen corroborate these from the 50 mg. portions. The 2- and 4-week determinations show a trifling gain of ammonia and considerable losses of nitrates. At 11 weeks there is an appreciable gain of ammonia, which is larger at the end of 15 weeks. The nitrate content remains low throughout all the sets, just as it did in Series I. It is, however, considered to be significant that with both of the larger amounts of kelp a distinct ammonification appeared at the same period, 11 weeks. This kelp had not been dried completely, as had that in Series I, a fact which has apparently considerable bearing on the availability of Macrocystis.

TABLE III.—*Series III, part 1. Effect of small and large amounts of dried Macrocystis pyrifera on the rate of ammonification and nitrification of dried blood in clay adobe soil from Berkeley, Cal.*

Material added to 200 gm. of soil.	Quantity of kelp added to 200 gm. soil. Gm.	Quantity of nitrogen added. Mg.	Quantity of soluble salts in kelp added. Gm.	Incubated 2 weeks.					Incubated 4 weeks.					Incubated 11 weeks.					Incubated 15 weeks.				
				Total quantity of nitrogen present in ammonia. Mg.	Average quantity of nitrogen gained in ammonia. Mg.	Total quantity of nitrogen present in nitrates. Mg.	Average quantity of nitrogen gained in nitrates. Mg.	Average percentage of nitrogen in blood changed to ammonia and nitrates.	Total quantity of nitrogen present in ammonia. Mg.	Average quantity of nitrogen gained in ammonia. Mg.	Total quantity of nitrogen present in nitrates. Mg.	Average quantity of nitrogen gained in nitrates. Mg.	Average percentage of nitrogen in blood changed to ammonia and nitrates.	Total quantity of nitrogen present in ammonia. Mg.	Average quantity of nitrogen gained in ammonia. Mg.	Total quantity of nitrogen present in nitrates. Mg.	Average quantity of nitrogen gained in nitrates. Mg.	Average percentage of nitrogen in blood changed to ammonia and nitrates.	Total quantity of nitrogen present in ammonia. Mg.	Average quantity of nitrogen gained in ammonia. Mg.	Total quantity of nitrogen present in nitrates. Mg.	Average quantity of nitrogen gained in nitrates. Mg.	Average percentage of nitrogen in blood changed to ammonia and nitrates.
Untreated soil				1.54		4.20			1.82		8.00			2.66		11.00			2.94		12.00		
						4.60			1.82		7.00			2.38		10.00			2.94		12.00		
Dried blood		10		2.94	0.70	12.00	7.60	83.00	1.82	0.07	14.00	7.00	69.30	2.52	0.14	15.00	4.50	46.40	2.24	.35	20.00	8.00	76.50
Do		100		16.25	14.92	52.00	47.60	62.52	1.68	.84	76.00	70.50	71.34	2.80	1.33	90.00	76.50	77.83	2.66	.14	90.00	75.00	74.86
				16.95		52.00					80.00			4.20		84.00			2.66		84.00		
Macrocystis pyrifera	1.11	10	0.396	2.10	.63	3.00	−1.45		2.52	.77	6.00	−1.50		3.22		19.00	−1.00		2.94	.14	9.00	−1.50	
Do	5.55	50	1.982	2.52	.98	2.92			2.52		6.00	−7.07		3.22		9.00			2.38		14.00		
Do	11.11	100	3.962	2.80	1.26	3.00			2.66	.63	.50	−7.25		3.50	.84	8.00	−2.50		2.94	.07	114.00	3.00	87.00
{ *Macrocystis pyrifera*	1.11	10	.396	2.52		.44	3.93		2.38	.49	.36			5.00		8.00	−10.03		2.80	8.40	116.00	11.48	107.10
{ Dried blood	1.11	10		2.66	.84	.50	4.21	57.43	2.24	.77	.30	4.50	52.70	5.60	.28	14.00	3.50	37.80	14.01		.60		None.
{ *Macrocystis pyrifera*	5.55	50	1.982	2.38		.20			2.52		12.00	5.98	None.	6.72	1.33	14.00	4.10	54.30	8.40	.70	20.00	8.00	87.00
{ Dried blood	1.11	10		3.92	2.73	.18	3.10	41.63	2.66	1.33	12.00	−7.28	None.	8.41	9.67	15.20	−10.18	None.	3.64	.21	20.00	10.50	107.10
{ *Macrocystis pyrifera*	11.11	100	3.962	4.90		7.00	−2.48		3.08	.63	1.52			2.94		14.00			3.36		22.50		None.
{ Dried blood	1.11	100		4.20	2.73	8.00	−4.30	2.50	2.24	1.40	.20	62.50	63.90	4.48	0.00	.24	69.50	69.50	2.52	.42	22.50	−10.25	65.93
{ *Macrocystis pyrifera*	5.55	50	1.982	17.37	15.83	1.92	41.60	None.	2.66		70.00			3.22	1.47	.40	59.50	60.97	3.78	−.07	1.00	66.00	52.94
{ Dried blood	11.11	100	3.962	17.65		1.92	−1.70	41.63	3.22		10.00	3.50	37.83	4.06		70.00			2.66		76.00	50.00	
{ *Macrocystis pyrifera*	5.55	50	1.982	44.34	43.39	.68	−4.20	38.67	30.43	34.33	70.00	3.50		3.92		70.00	−10.25	40.61	2.94	2.94	80.00	66.00	65.93
{ Dried blood	11.11	100	3.962	45.81	42.87	.12			35.87	23.40	10.00	−7.08	16.32	51.70	50.86	.30			3.36		60.00	50.00	52.94
				53.10		.16			27.32		12.00			55.06		.20			8.13		64.00		
				36.10		.15			33.12		.50					.35			35.16	2.94	20.00	1.00	39.59
											.35								47.03	38.59	6.00		

TABLE IV.—Series III, part 2. Effect of small and large amounts of dried *Nereocystis luetkeana* on the rate of ammonification and nitrification of dried blood in clay adobe soil from Berkeley, Cal.

Material added to 200 gm. of soil.	Quantity of kelp added to 200 gm. soil. (Gm.)	Quantity of nitrogen added. (Mg.)	Quantity of soluble salts in kelp added. (Gm.)	Incubated 2 weeks. Total quantity of nitrogen present in am-monia. (Mg.)	Average quantity of nitrogen gained in am-monia. (Mg.)	Total quantity of nitrogen present in nitrates. (Mg.)	Average quantity of nitrogen gained in nitrates. (Mg.)	Percentage of total nitrogen gained, all cal-culated to dried blood present.	Percentage of *Nereo-cystis luetkeana* nitrogen gained.	Incubated 4 weeks. Total quantity of nitrogen present in am-monia. (Mg.)	Average quantity of nitrogen gained in am-monia. (Mg.)	Total quantity of nitrogen present in nitrates. (Mg.)	Average quantity of nitrogen gained in nitrates. (Mg.)	Percentage of total nitrogen gained, all cal-culated to dried blood present.	Percentage of *Nereo-cystis luetkeana* nitrogen gained.
Untreated soil				1.54 / 1.82		4.20 / 4.60				1.82 / 1.82		8.00 / 7.00			
Dried blood		10		2.94 / 1.82	0.70	12.00 / 12.00	7.60	83.00		1.82 / 1.68	−0.07	14.00 / 15.00	7.00	69.30	
Do		100		16.25 / 16.95	14.92	52.00 / 52.00	47.60	62.52		2.52 / 2.80	.84	76.00 / 80.00	70.50	71.34	
Nereocystis luetkeana	0.59	10	0.273	1.82 / 4.34	1.40	7.60 / 7.00	3.20		46.00	2.94 / 2.80	1.05	10.00 / 9.60	2.30		33.50
Do	2.94	50	1.366	14.71 / 16.39	13.87	7.00 / 7.00	2.60		32.94	6.02 / 6.58	4.48	12.00 / 13.00	5.00		18.96
Do	5.88	100	2.732	26.90 / 26.20	24.87	3.80 / 3.40	−.80		24.07	28.72 / 34.47	29.77	5.00 / 4.00	−3.00		26.77
{*Nereocystis luetkeana* / Dried blood}	.59	10	.273	1.96 / 2.24	.42	12.40 / 12.40	8.00	84.20	1.20	2.80 / 3.08	1.12	16.00 / 16.00	8.50	96.20	26.90
{*Nereocystis luetkeana* / Dried blood}	2.94	50	1.366	13.45 / 12.33	11.21	9.00 / 10.00	5.10	163.10	16.02	7.28 / 11.21	7.42	20.00 / 18.00	11.50	189.20	23.98
{*Nereocystis luetkeana* / Dried blood}	5.88	100	2.732	30.82 / 30.54	29.00	3.60 / 3.60	−.80	282.00	19.90	28.72 / 35.73	30.42	4.00 / 4.10	−3.45	269.7	20.04
{*Nereocystis luetkeana* / Dried blood}	.59	10	.273	12.89 / 18.49	14.01	50.00 / 48.00	44.60	58.61	None	2.52 / 2.80	.84	70.00 / 68.00	61.50	62.34	None.
{*Nereocystis luetkeana* / Dried blood}	2.94	50	1.366	53.80 / 56.60	53.52	20.00 / 14.00	12.60	66.12	3.60	38.53 / 40.91	37.90	30.00 / 31.00	23.00	60.90	None.
{*Nereocystis luetkeana* / Dried blood}	5.88	100	2.732	83.36 / 78.74	79.37	4.00 / 4.00	−.40	78.97	16.45	91.77 / 85.74	86.93	6.00 / 5.00	2.00	88.93	17.59

The data obtained where dried blood alone was added do not call for any special comment. The production of ammonia and nitrates, with some minor variations, proceeds through each period. When Macrocystis and blood are present, the conditions are very different. The blood is a very available material; the Macrocystis is slowly available. It is therefore reasonable to suppose that the first gains of ammonia and nitrates will be due to the blood. Later, the Macrocystis may also furnish a portion of the nitrogen gained. If at all times the percentage of total gain is calculated as if it were due to the nitrogen of the blood, it can readily be seen whether the total amount gained is greater or less than that where the blood alone was present. This has accordingly been done. If the kelp also furnishes a large part of the gain, the result will evidently exceed 100 per cent and will clearly indicate this fact. It is not claimed that this procedure is strictly accurate, but it will demonstrate whether as much nitrogen is gained with blood and kelp as with blood alone.

With two weeks' incubation we find that this is not so. All quantities of Macrocystis give a reduction in the total gain. Ten mg. of nitrogen in kelp added to one hundred in blood show the smallest inhibitive effect. At four weeks the results are practically the same. Eleven weeks' incubation shows a distinct reduction of the inhibition; especially is this so in regard to nitrification. At 15 weeks it is found that with 10 mg. of blood and with both 10 and 50 mg. of kelp inhibition has ceased and there has been a gain from the kelp added. With the 100-mg. tumblers of blood and Macrocystis nitrification is observed to have commenced. The general evidence would appear to be that at first there undoubtedly is an inhibitive effect from the Macrocystis. This inhibition is gradually reduced as time goes on.

The results from the two sets of tumblers with dried and ground Nereocystis furnish a very striking contrast to those from the Macrocystis. With the kelp alone there is a very ready and uniform conversion to both ammonia and nitrates. It is only with the 100-mg. portions that a very marked inhibition of nitrification has occurred.

The total gain of ammonia and nitrates has again been calculated as if it were all due to blood. The resulting percentages in a number of cases show a greater gain than occurred where blood alone was added to the soil. The difference is naturally assumed to be due to the Nereocystis present. This difference between the blood alone and blood plus Nereocystis has been calculated as the percentage of Nereocystis nitrogen gained. The data throughout show that the Nereocystis is a very available material. This corroborates the results obtained in Series I, which showed that it readily ammonified. We have now proved in addition that it also nitrifies rapidly.

SERIES IV.—COMPARATIVE AMMONIFICATION AND NITRIFICATION OF MACROCYSTIS, FRESH, AIR-DRY, HIGHLY HEATED, AND PARTIALLY DRY

The results of Series III showed that the air-dry Macrocystis with which we are principally concerned is a slowly available material. Series I and II indicated that the kelp which had been dried to a constant weight for a number of hours was less available. It therefore appeared desirable to find what degrees of availability there would be between the thoroughly dry kelp and the fresh material. It was quite possible that this might throw some light on the most desirable method of handling the kelp commercially. One patent has been taken out for a process by which the kelp is heated to from 250° to 270° C. and thoroughly "parched." Many advantages for this process are claimed.

A series of experiments were therefore conducted at Pacific Grove. In these *Macrocystis pyrifera* was air-dried completely, so that it contained only 8.68 per cent of moisture. Duplicate portions were dried so that they contained 28.63, 37.15, and 55.32 per cent of moisture. Fresh kelp was also gathered from the same bed. On returning to Berkeley, two duplicate portions of the air-dry kelp of 500 gm. each were heated to from 250° to 270° C. till thoroughly parched. During this process 36 per cent of the organic matter present was lost, and in this organic matter driven off 31 per cent of the nitrogen originally present in the kelp was also lost.

The materials were all used in an ammonification and nitrification study which was conducted for three weeks. The soil used was clay adobe, as in Series III. Two portions of each sample of kelp were added to triplicate sets of tumblers. One weight of kelp used contained just 0.27 gm. of soluble salts, while the other contained just twice as much, 0.54 gm. (See Table V.)

It will be observed that the fresh kelp as before gave a very satisfactory conversion to ammonia and nitrates. The air-dry Macrocystis with the smaller quantity added gave a negative result. With double the amount of kelp there was a small but consistent gain. The parched kelp gave no conversion whatsoever. The partially dry kelp was all fairly available. That which contained 28 per cent of moisture was, in fact, more readily changed than the fresh sample. The evident conclusion is that Macrocystis will be more readily decomposed if it can be handled without drying completely. It should be noted that the samples with 28 and 37 per cent of moisture could be kept without danger of decay or mold. That with 55 per cent was too moist to be safely stored.

TABLE V—(*Series IV*).   Ammonification and nitrification of *Macrocystis pyrifera, fresh, air-dry, highly heated, and partially dry.   Incubated for three weeks*

Material added to 200 gm. of soil.	Percentage of moisture in kelp.	Quantity of kelp added.	Quantity of nitrogen added.	Quantity of soluble salts in kelp added.	Quantity of nitrogen gained in ammonia.	Quantity of nitrogen gained in nitrates.	Quantity of nitrogen gained in ammonia and nitrates.	Average percentage of added nitrogen gained in ammonia and nitrates.
		Gm.	Mg.	Gm.	Mg.	Mg.	Mg.	
Macrocystis pyrifera, fresh............	87.00	7.00	19.45	0.27	0.23 / .23 / −.05	5.14 / 3.94 / 2.59	5.37 / 4.17 / 2.54	20.67
Do.............	87.00	14.00	38.90	.54	.37 / .51 / −.05	9.27 / 11.27 / 11.27	9.64 / 11.78 / 11.22	27.97
Macrocystis pyrifera, air-dry............	8.68	.72	10.35	.27	.65 / .65 / .51	−1.56 / −1.56 / −1.56	−.91 / −.91 / −1.05	None.
Do.............	8.68	1.44	20.70	.54	.65 / .79 / 1.35	.94 / .94 / .44	1.59 / 1.73 / 1.79	8.21
Macrocystis pyrifera, heated to 250° C....	1.44	.50	8.43	.27	−.19 / .65 / .23	−1.56 / −1.56 / −2.06	−1.75 / −.91 / −1.83	None.
Do.............	1.44	1.00	16.86	.54	.09 / .51 / .09	−1.56 / −2.06 / −2.06	−1.47 / −1.55 / −1.97	None.
Macrocystis pyrifera..	28.63	.99	12.32	.27	.23 / −.05 / .23	3.44 / 3.44 / 3.44	3.67 / 3.39 / 3.67	28.98
Do.............	28.63	1.98	24.64	.54	2.19 / 1.07 / .37	5.94 / 5.94 / 5.94	8.13 / 7.01 / 6.31	29.02
Do.............	37.15	1.09	10.73	.27	.51 / .09 / .65	.94 / .94 / .94	1.45 / 1.03 / 1.59	15.58
Do.............	37.15	2.19	21.46	.54	.37 / 1.35 / .23	4.94 / 5.94 / 4.94	5.31 / 7.29 / 5.17	27.59
Do.............	55.32	1.34	10.20	.27	.09 / .79 / .37	1.94 / 1.94 / 1.94	2.03 / 2.73 / 2.31	23.04
Do.............	55.32	2.68	20.40	.54	1.07 / .37 / 1.21	3.44 / 4.94 / 3.44	4.51 / 5.31 / 4.65	24.12

GENERAL DISCUSSION OF RESULTS

The preceding studies on the availability of the major kelps of the Pacific coast demonstrate a number of important facts, which are consistently shown by all the series of experiments. *Nereocystis luetkeana*, which is not commercially important, proved to be the most available. It is not a highly nitrogenous substance, like dried blood or cottonseed meal. We should not, therefore, expect it to be so readily decomposed as these materials. The rate of ammonification and nitrification which it has shown in Series I and III is therefore considered to be very satisfactory.

Pelagophycus porra ranks next to the Nereocystis in availability, while *Macrocystis pyrifera*, the commercial variety, is the least available of all. The studies carried through with this material prove that when very completely oven-dried this kelp is changed in the soil with extreme slowness. When sun-dried so that it can be readily ground, it requires about 11 weeks to ammonify and to begin to nitrify appreciably. In Series IV a slight gain is shown by one quantity of air-dry Macrocystis at the end of three weeks. This kelp was dried for the shortest possible time in the sun and the drying was then completed in the incubator room at 28° C. The other samples in Series IV, which contained greater amounts of moisture, were all more available. It would therefore appear that Macrocystis should be dried and ground at as low a temperature as possible. Commercially, artificial heat will probably be necessary for drying. This drying will have to continue till the kelp is crisp and practically water-free, but should not be carried on for a longer time than may be necessary to have it attain this condition.

The addition of moderate quantities of Nereocystis to the soil has not caused any great inhibition to either ammonification or nitrification. With Macrocystis, however, very appreciable inhibition is at first shown. As time goes on, this is gradually less evident. With the smaller amounts of kelp used in field fertilization, the inhibition would undoubtedly be less and would probably be sooner counteracted.

CONCLUSIONS

(1) In preparing dried and ground kelp as a fertilizer the availability or readiness with which the nitrogen in it is changed to ammonia and nitrates in fresh field soil was found to vary with different species and with the manner of preparation.

(2) The nitrogen of *Nereocystis luetkeana* is relatively very available, while that of *Pelagophycus porra* is less readily changed. These two varieties are of minor commercial importance.

(3) *Macrocystis pyrifera*, the commercial variety, is very slowly changed in the soil.

(4) The availability of the nitrogen of *M. pyrifera* is greatest when the kelp is added in a fresh or only partially dried condition.

(5) The availability of its nitrogen decreases materially when Macrocystis is fully dried.

(6) Removing a large portion of the salts from either fresh or dry Macrocystis by leaching does not cause it to decompose more readily.

(7) Macrocystis must be dried till crisp in order to grind readily. This drying should not be continued longer than necessary, and the kelp should not be scorched or overheated.

(8) The addition of moderate quantities of Nereocystis to a sample of fresh soil in the laboratory did not cause any great interference with

either ammonification or nitrification of readily available organic matter, such as dried blood.

(9) Similar experiments with Macrocystis showed at first a decrease in the rate of transformation, especially in nitrification. This decrease did not continue and as time passed the ammonification and nitrification became practically normal.

(10) When using kelp in field practice, it is probable there would be no interference with either ammonification or nitrification from either the kelp or the salts present in it.

LITERATURE CITED

(1) AMERICAN PUBLIC HEALTH ASSOCIATION.
 1912. Standard Methods for the Examination of Water and Sewage. ed. 2f
  144 p. New York.

(2) BOUSSINGAULT, ————.
 1838. Recherches chimiques sur la végétation, entreprises dans le but d'examiner si les plantes prennent de l'azote à atmosphère. *In* Ann. Chim. et Phys., t. 67, p. 5–54.

(3) BROWN, P. E.
 1911. Some bacteriological effects of liming. Iowa Agr. Exp. Sta. Research Bul. 2, p. 51–107, illus.

(4) ————
 1912. Bacteriological studies of field soils.—I. The effects of lime. Iowa Agr. Exp. Sta. Research Bul. 5, p. 191–210.

(5) BURD, J. S.
 1915. The economic value of Pacific coast kelps. Cal. Agr. Exp. Sta. Bul. 248, p. 183–215, 3 fig.

(6) BURGESS, P. S.
 1913. The aluminum reduction method as applied to the determination of nitrates in "alkali" soils. *In* Univ. Cal. Pub. Agr. Sci., v. 1, no. 4, p. 51–62, 1 fig.

(7) LIPMAN, C. B.
 1909. Toxic and antagonistic effects of salts as related to ammonification by Bacillus subtilis. *In* Bot. Gaz., v. 48, no. 2, p. 105–125, 5 fig.

(8) ————
 1910. On physiologically balanced solutions for bacteria (B. subtilis). *In* Bot. Gaz., v. 49, no. 3, p. 207–215.

(9) ————
 1910. On the lack of antagonism between calcium versus magnesium and also between calcium versus sodium. *In* Bot. Gaz., v. 49, no. 1, p. 41–50, 2 fig.

(10) ————
 1913. Antagonism between anions as affecting ammonification in soils. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 36, No. 15/18, p. 382–394, 3 fig.

(11) ———— and BROWN, P. E.
 1909. Notes on methods and culture media. *In* N. J. Agr. Exp. Sta. 29th Ann. Rpt. [1907]/08, p. 129–136.

(12) ———— and BURGESS, P. S.
 1914. Antagonism between anions as affecting soil bacteria. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 41, No. 11/17, p. 430–444, 6 fig.

(13) LOEB, Jacques.
    1900. On ion-proteid compounds and their rôle in the mechanics of life phe-
        nomena.—I. The poisonous character of a pure NaCl solution. *In*
        Amer. Jour. Physiol., v. 3, no. 7, p. 327–338.
(14) ———
    1900. On the different effect of ions upon myogenic and neurogenic rhyth-
        mical contractions and upon embryonic and muscular tissue. *In*
        Amer. Jour. Physiol., v. 3, no. 8, p. 383–396.
(15) LÖHNIS, Felix.
    1910. Handbuch der landwirtschaftlichen Bakteriologie. 907 p. Berlin.
(16) OSTERHOUT, J. V.
    1908. Die Schutzwirkung des Natriums für Pflanzen. *In* Jahrb. Wiss. Bot.
        [Pringsheim], Bd. 46, Heft 2, p. 121–136, 3 fig.
(17) ———
    1909. On similarity in the behavior of sodium and potassium. *In* Bot. Gaz.,
        v. 48, no. 2, p. 98–104, 4 fig.
(18) STEVENS, F. L., and WITHERS, W. A.
    1908. Concerning the difference of behavior of soil organisms when in solutions
        and when in soils. *In* Science, n. s., v. 27, no. 704, p. 991.
(19) ——— ——— et al.
    1909. Studies in soil bacteriology.—I. Nitrification in soils and in solutions.
        In Centbl. Bakt. [etc.], Abt. 2, Bd. 23, No. 10/13, p. 355–373.
(20) ——— ———
    1910. Studies in soil bacteriology.—IV. The inhibition of nitrification of
        organic matter, compared in soils and in solutions. *In* Centbl. Bakt.
        [etc.], Abt. 2, Bd. 27, No. 4/9, p. 169–186.

ORGANIC CONSTITUENTS OF PACIFIC COAST KELPS

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INTRODUCTION AND PLAN OF WORK

The giant kelps of the Pacific coast have been regarded during recent years as commercially profitable sources of potash and iodin. The high content of these constituents in the kelp was first given prominence by Balch (1),[1] and later the Bureau of Soils of the United States Department of Agriculture (4) made further studies and mapped out many of the beds. These investigations were followed by a widespread interest in kelps and it was the prevailing idea that these plants would furnish the raw material for industries of considerable magnitude. It seemed, however, that such predictions required further verification through more extended chemical studies than were available, since in many directions exact information was entirely lacking. Accordingly the Chemical Laboratory of the California Experiment Station during the past year has carried on a general investigation of the subject the principal results of which are discussed in publications of this Station by Burd (3) and Stewart (32).

While the potash and iodin values have, as a matter of course, received first attention in all discussions of a kelp industry, it has been apparent that any commercially valuable by-products of an organic nature would greatly enhance the possibilities of utilizing kelp with a margin of profit. Practically no studies of the organic constituents of the California kelps have been made prior to the writer's, and it is with this aspect of the investigations that the present paper deals.[2] It is not the intention to regard the experiments herein described as forming in any sense a complete and final study of the numerous questions involved. The composition and chemical physiology of these marine algæ are in many respects unique. There are obviously infinite possibilities for studies of great scientific interest along these lines. If opportunity is afforded, more detailed experiments in regard to the nature of certain interesting constituents of the kelp will be conducted in this laboratory. It is, however, deemed advisable to present such results and conclusions as are now at hand, owing to the considerable interest recently manifested in this subject.

The conceivable uses for the organic matter of kelp which have most frequently been mentioned include destructive distillation, feeding of

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[1] Reference is made by number to "Literature cited," p. 56–58.

[2] Acknowledgment is made to Mr. W. H. Dore, of the Chemical Laboratory of the California Experiment Station, for much assistance in the analytical work and suggestions as to methods.

animals, production of sizes, glues, or varnishes, clarification of wines, and paper making. In these studies on the kelp the writer has endeavored to obtain analytical and other data essential to a more accurate judgment in regard to the above possibilities, at the same time having in mind certain factors of purely theoretical interest. For greater convenience of discussion the work is described under the following subheads: General study of important chemical groups; Forms of nitrogen; Carbohydrates; Cellulose; Hydrolysis; Sulphur content; Forms of iodin; Economic considerations, including the results of destructive distillation.

Several varieties of seaweeds have been utilized commercially in Japan for over two centuries. They have served as a basis for the preparation of "Kombu" and other foodstuffs, for glue manufacture, and for the manufacture of "Kanten," in extensive use under the name "agar-agar" (22). The commercially important seaweeds of Japan are, however, of different species and undoubtedly have chemical properties distinct from those of the giant kelps (*Macrocystis* spp. and *Nereocystis* spp.) of the Pacific coast.

The earliest chemical studies of seaweed were made by Stanford (24–31) on European species about 40 years ago. He described a product obtained from kelp, which he called "algin" or "alginic acid," and for which he claimed remarkable properties. Many uses were suggested for this substance: A sizing material for fabrics, a glue, a food, a water-softening reagent, and various other applications. A theoretical formula was deduced for this so-called "algin," and its elementary composition was stated, but Stanford presented no systematic or detailed chemical studies in support of his conclusions. Furthermore the chemical methods available at that period were very imperfect.

Since the various papers of Stanford (24–31) were published, only occasional articles of very limited scope have appeared on the organic chemistry of the important marine algæ. For the giant kelp of the Pacific coast no systematic analyses or chemical studies of the organic matter have been made, although there are many assertions regarding the presence of valuable substances, such as rubber, useful varnishes, or commercially profitable distillation products. These points will be discussed under their appropriate headings.

GENERAL STUDY OF IMPORTANT CHEMICAL GROUPS

The limitations in value of the so-called proximate analyses by the "official" methods are recognized. Such data at present seem, nevertheless, to be essential for purposes of general classification and comparison. In the accompanying tables an extended series of such analyses is presented. The following species of kelp were investigated: *Macrocystis pyrifera, Nereocystis luetkeana, Pelagophycus porra, Egregia laevigata, Egregia menziesii, Laminaria andersonii, Iridaea* sp. Analyses were made

on the analytical samples collected by Mr. Guy R. Stewart, of this laboratory. Every precaution was taken to insure fair and representative samples, as described in the preceding paper (32). Tabulations are made on the water-free basis and also on the original material. In the consideration of possible utilization of kelp products proper emphasis should be given to the high percentage of water in the plant.

A qualitative examination of the varieties of kelp investigated indicates the presence of very complex compounds in a highly colloidal state. Starch and soluble reducing sugars are absent from these plants. In all cases strong tests for furfural (pentosan test) are given, and some samples gave the galactan test by oxidation with nitric acid. The major portion of the total organic matter present is insoluble in water and in alcohol. These relations are given in Table I.

TABLE I.—*Organic matter in kelp*

No. of sample.	Species of kelp and part used.	Percentage of total organic matter	Percentage of plant.		Percentage of total organic matter.	
			Water-soluble organic matter.	Alcohol-soluble organic matter.	Water-soluble organic matter.	Alcohol-soluble organic matter.
42	*Egregia* spp.................	67. 87	10. 08	6. 84	14. 9	10. 1
43	*Laminaria andersonii*...........	73. 80	23. 32	8. 56	31. 6	11. 6
44	*Iridaea* sp....................	69. 12	. 25. 80	8. 16	37. 3	11. 8
45. 1	*Nereocystis luetkeana*, leaves......	47. 27	18. 88	12. 72	39. 9	26. 9
45. 2	*Nereocystis luetkeana*, stems.......	39. 02	13. 24	6. 68	33. 9	17. 1
46. 1	*Macrocystis pyrifera*, leaves.......	54. 30	11. 56	8. 36	21. 3	15. 4
46. 2	*Macrocystis pyrifera*, stems........	51. 22	25. 40	13. 12	49. 6	25. 6
47. 1	*Pelagophycus porra*, leaves.......	58. 60	23. 32	18. 04	39. 8	30. 8
47. 2	*Pelagophycus porra*, stems........	37. 35	14. 40	9. 28	38. 5	24. 9

It may be noted in this connection that certain varieties of marine algæ, investigated in other parts of the world, have shown different chemical characteristics. Levulose, mannit, starch, and easily hydrolyzable polysaccharids have been reported (2, 18).

Table II gives the general composition of the various portions of the kelp plants for the different species investigated. The extent of the variations between individual plants is indicated in Table III. The total organic matter varies from one-third to three-fourths of the dry weight. Using the conventional factor of 6.25 for nitrogen, we have as high as 17 per cent of protein if all the nitrogen existed in that form. (The forms of nitrogen will be discussed later.) The plants of Macrocystis obtained at Pacific Grove are distinctly higher in this constituent, as compared with the samples of Macrocystis from the vicinity of San Diego. In none of the species is the percentage of ether extract important, the average exceeding 1 per cent in only the one case of Nereocystis

leaves.   A considerable portion of the ether extract is, of course, coloring matter and not true fat.   The percentages of crude fiber are not high, from 6 to 10 per cent being fairly constant for all samples.   The same statement applies to the pentosans.   *Iridaea* sp. (a rockweed) is exceptional in this respect, having less than 1 per cent as pentosans.   Numerous attempts were made to estimate by the mucic-acid method the proportion of galactans present, but in most cases entirely untrustworthy results were obtained.   The method was found to be inherently unreliable and especially unadaptable to materials of this type.   Approximate results were obtained for the Pelagophycus and the Iridaea, the former yielding mucic acid equivalent to 3 per cent of galactan, the latter 10 per cent.

TABLE II.—*Average composition of the organic matter of Pacific coast kelp, calculated on whole plant*

Material used.	Number of samples analyzed.	Moisture.	Percentage composition calculated to fresh material.								Percentage composition calculated to a water-free basis.							
			Total salts.	Total organic matter.	Nitrogen, 6.25.	Ether extract.	Crude fiber.	Pentosans.	Water-soluble alcohol precipitate.	Sodium carbonate soluble acid precipitate (algin).	Total salts.	Total organic matter.	Nitrogen, 6.25.	Ether extract.	Crude fiber.	Pentosans.	Water-soluble alcohol precipitate.	Sodium carbonate soluble acid precipitate (algin).
Macrocystis pyrifera, harvestable portion, San Diego	8	86.3	5.24	8.45	1.02	0.046	0.98	1.06	0.91	2.56	38.2	61.7	7.4	0.34	7.2	7.7	6.6	18.7
Macrocystis pyrifera, harvestable portion, Pacific Grove	4	87.7	5.28	7.00	1.65	.049	.93	.77	1.07	1.77	42.9	56.9	13.4	.40	7.6	6.3	8.7	14.4
Macrocystis pyrifera, nonharvestable portion, San Diego	8	87.7	5.16	7.11	1.33	.054	.89	.98	1.08	2.15	42.0	57.8	10.8	.44	7.2	8.0	8.8	17.5
Nereocystis luetkeana, entire plant, Pacific Grove	4	91.7	4.52	3.74	.90	.088	.42	.53	.71	1.17	54.5	45.1	10.8	1.06	5.1	6.4	8.6	14.1
Pelagophycus porra, entire plant, San Diego	2	89.7	5.43	4.91	.77	.028	.64	.87	.60	1.66	52.7	47.7	7.5	.27	6.2	8.4	5.8	16.1
Egregia laevigata, entire plant, San Diego	2	83.7	5.78	10.52	1.89	.144	1.47	1.60	1.48	3.04	35.5	64.5	11.6	.88	9.0	9.8	9.1	18.7
Egregia menziesii, entire plant, Pacific Grove	3	83.6	5.48	10.88	2.82	.110	1.43	1.48	.90	3.14	33.4	66.3	17.2	.67	8.7	9.0	5.5	19.1
Laminaria andersonii, entire plant	1	78.5	5.70	15.80	3.22	.140	2.24	2.15	.37	4.90	26.5	73.5	15.0	.65	10.4	10.0	1.7	22.8
Iridaea sp., entire plant	1	80.1	6.25	13.70	3.38	.087	2.09	.18	.26	.20	31.4	68.8	17.0	.44	10.5	.9	1.3	1.0

TABLE III.—*Maximum, minimum, and average composition of organic matter of leaves and stems of Pacific coast kelp*

Material used	Number of samples	Percentage composition calculated to fresh material.									Percentage composition calculated to a water-free basis.							
		Moisture.	Total salts.	Total organic matter.	Nitrogen, 6.25.	Ether extract.	Crude fiber.	Pentosans.	Water-soluble alcohol precipitate.	Sodium carbonate soluble acid precipitate (algin).	Total salts.	Total organic matter.	Nitrogen, 6.25.	Ether extract.	Crude fiber.	Pentosans.	Water-soluble alcohol precipitate.	Sodium carbonate soluble acid precipitate (algin).
Macrocystis pyrifera, maximum composition, harvestable leaves, San Diego and La Jolla.	8	88.0	6.26	11.99	1.75	0.065	1.37	1.38	1.06	3.72	49.9	71.4	10.8	0.43	9.0	9.1	7.0	24.5
Macrocystis pyrifera, minimum composition, harvestable leaves, San Diego and La Jolla.	8	83.2	4.44	7.22	.87	.035	.72	1.05	.55	1.97	30.4	60.2	5.2	.23	6.0	6.5	3.7	16.4
Macrocystis pyrifera, average composition, harvestable leaves, San Diego and La Jolla.	8	85.3	5.22	9.50	1.27	.045	1.04	1.13	.73	2.71	35.5	64.5	8.6	.31	7.1	7.7	5.0	18.4
Macrocystis pyrifera, average composition, harvestable leaves, Pacific Grove and vicinity.	4	86.8	5.33	7.87	2.19	.046	1.07	.84	.90	1.80	40.4	59.6	16.6	.35	8.1	6.4	6.8	13.6
Macrocystis pyrifera, maximum composition, harvestable stems, San Diego and La Jolla.	8	88.2	5.99	7.73	.92	.061	1.07	1.05	1.55	2.66	45.4	61.8	8.1	.55	8.1	8.7	12.4	22.0
Macrocystis pyrifera, minimum composition, harvestable stems, San Diego and La Jolla.	8	85.6	4.78	5.63	.34	.033	.71	.91	.53	1.92	42.3	49.8	2.6	.30	7.2	8.2	4.4	17.1
Macrocystis pyrifera, average composition, harvestable stems, San Diego and La Jolla.	8	88.2	5.29	6.48	.55	.049	.85	.94	1.24	2.28	44.8	54.9	4.7	.42	7.2	8.0	10.5	19.3
Macrocystis pyrifera, average composition, harvestable stems, Pacific Grove and vicinity.	4	89.3	5.20	5.52	.74	.054	.69	.65	1.35	1.72	48.6	51.6	6.9	.51	6.5	6.1	12.6	16.1
Macrocystis pyrifera, average composition, nonharvestable leaves, San Diego and La Jolla.	6	86.9	5.54	7.56	1.95	.056	.94	.94	.95	2.11	42.3	57.7	14.9	.43	7.2	7.2	7.3	16.1
Macrocystis pyrifera, average composition, nonharvestable leaves, Pacific Grove and vicinity.	7	86.5	5.51	8.03	2.14	.063	.74	.79	.53	1.75	40.8	59.5	15.9	.47	5.5	5.9	3.9	13.0
Macrocystis pyrifera, average composition, nonharvestable stems, San Diego and La Jolla.	6	88.4	4.83	6.73	.80	.051	.86	1.01	1.18	2.18	41.6	58.0	6.9	.44	7.4	8.7	10.2	18.8
Macrocystis pyrifera, average composition, nonharvestable stems, Pacific Grove and vicinity.	2	88.6	5.08	6.27	.75	.051	.75	.71	.96	1.84	44.6	55.0	6.6	.45	6.6	6.2	8.4	16.1

	No.																	
Nereocystis leutkeana, average composition, leaves, Pacific Grove and vicinity	4	91.3	4.40	4.27	1.09	.126	.41	.55	.76	1.25	50.6	49.1	12.5	1.45	4.7	6.3	8.7	14.4
Nereocystis luetkeana, average composition, stems, Pacific Grove and vicinity	4	92.4	4.70	2.88	.57	.025	.44	.49	.64	1.03	61.8	37.9	7.5	.33	5.8	6.5	8.4	13.6
Pelagophycus porra, average composition, leaves, San Diego and vicinity	2	88.0	5.70	6.25	.99	.034	.73	.99	.55	1.93	47.5	52.1	8.2	.28	6.1	8.2	4.6	16.1
Pelagophycus porra, average composition, stems, San Diego and vicinity	2	91.8	5.06	3.14	.49	.023	.52	.72	.67	1.30	61.7	38.3	6.0	.28	6.3	8.8	8.2	15.9
Egregia laevigata, average composition, complete, San Diego and vicinity	2	83.7	5.78	10.52	1.89	.144	1.47	1.60	1.48	3.04	35.5	64.5	11.6	.88	9.0	9.8	9.1	18.7
Egregia menziesii, average composition, complete, Pacific Grove and vicinity	3	83.6	5.48	10.88	2.82	.110	1.43	1.48	.90	3.14	33.4	66.3	17.2	.07	8.7	9.0	5.5	19.1
Laminaria andersonii, composition, complete	1	78.5	5.70	15.80	3.22	.140	2.24	2.15	.37	4.90	26.5	73.5	15.0	.65	10.4	10.0	1.7	22.8
Iridaea spp., composition, complete	1	80.1	6.25	13.70	3.38	.087	2.09	.18	.26	.20	31.4	68.6	17.0	.44	10.5	.9	1.3	1.0

FORMS OF NITROGEN PRESENT

In order to estimate accurately the value of nitrogenous substances, more than a mere statement of the percentage of nitrogen is necessary. All compounds of nitrogen are not of equal worth, especially in nutrition. Experiments were therefore undertaken to determine the general forms of nitrogen present in the kelp. Water-soluble nitrogen, alcohol-soluble nitrogen, and nonprotein nitrogen by Stutzer's reagent, also by phosphotungstic acid, were estimated. Extractions were made on dried and finely ground samples. An important part of the nitrogen (from one-fifth to one-third) is soluble in cold water. (See Table IV.) About the same proportion is classed as nonprotein nitrogen, using the methods just mentioned. Other experiments, in which fresh kelp was leached, showed similar relations for soluble and insoluble nitrogen. The water extracts from a number of samples of ground kelp were analyzed for acid amid and ammonia nitrogen, using the method of Abderhalden. Protein nitrogen was precipitated by means of phosphotungstic acid, the filtrate boiled in an 8 per cent solution of hydrochloric acid and distilled in the presence of an excess of magnesium oxid. In all cases the amount of nitrogen so estimated was insignificant.

TABLE IV.—*Forms of nitrogen in kelp*

No.	Material used.	Percentage of dried and ground sample.					Percentage distribution of nitrogen.			
		Total nitrogen.	Nonprotein nitrogen.		Water-soluble nitrogen.	Alcohol-soluble nitrogen.	Nonprotein nitrogen.		Water-soluble nitrogen.	Alcohol-soluble nitrogen.
			By Stutzer's reagent.	By phosphotungstic acid.			By Stutzer's reagent.	By phosphotungstic acid.		
1.1	*Macrocystis pyrifera* No. 1, harvestable leaves	1.69	0.55	0.42	0.58	0.18	32.5	24.9	34.3	10.7
1.2	*Macrocystis pyrifera* No. 1, harvestable stems	.98	.30	.32	.38	.12	30.6	32.7	38.8	12.2
1.3	*Macrocystis pyrifera* No. 1, nonharvestable leaves	2.40	.89	.70	.99	.32	37.1	29.2	41.2	13.3
9.1	*Macrocystis pyrifera* No. 9, harvestable leaves	1.01	.19	.18	.20	.06	18.8	17.8	19.8	6.0
9.3	*Macrocystis pyrifera* No. 9, nonharvestable leaves	1.62	.32	.25	.41	.10	19.8	15.4	25.3	6.2
15.1	*Pelagophycus porra*, No. 4, leaves	1.24	.22	.18	.23	.18	17.7	14.5	18.5	14.5
15.2	*Pelagophycus porra* No. 4, harvestable stem	.98	.15	.16	.23	.07	15.3	16.3	23.5	7.1
27.1	*Nereocystis luetkeana* No. 1, leaves	1.86	.50	.37	.52	.35	26.9	19.9	28.0	18.8
28.1	*Nereocystis luetkeana* No. 2, leaves	1.90	.55	.48	.58	.43	29.0	25.3	30.5	22.6
29.1	*Nereocystis luetkeana* No. 3, leaves	2.20	.65	.57	.73	.47	29.5	25.9	33.2	21.3
29.2	*Nereocystis luetkeana* No. 3, stem	1.05	.24	.21	.28	.15	22.9	20.0	26.7	14.3
30.1	*Nereocystis luetkeana* No. 4, leaves	1.97	.48	.45	.55	.32	24.4	22.8	27.9	16.2
42	*Egregia menziesii* No. 3, complete strands	2.75	.43	.37	.48	.24	15.6	13.5	17.5	8.7
43	*Laminaria andersonii*, entire plants	2.38	.48	.41	.65	.32	20.2	17.2	27.3	13.4
44	*Iridaea* spp., entire plants	2.67	.52	.53	.58	.36	19.5	19.8	21.7	13.5

CARBOHYDRATES IN KELP

Carbohydrates or analogous bodies make up the principal portion of the organic matter. The carbohydrates of these algæ are complex colloidal substances which would ordinarily be classified among the vegetable gums, or pectins. Very little information is obtainable for these groups, and there are no satisfactory specialized chemical methods available for their study. In general, complex mucilaginous polysaccharids are characteristic of marine algæ (8, T. 1, p. 68), replacing the starch, cellulose, and simple sugars of most land plants. Even where starch and simple carbohydrates have been reported to be present in algæ, the amounts are relatively small. The physical properties form the most important consideration in the utilization of the carbohydrates of the algæ. From some products valuable jellies may be prepared, for example, agar-agar. The California kelps studied in this laboratory do not have this property of jellification to any valuable degree.

ALGIN

The fraction to which the name "algin" has been given is quantitatively and in point of interest the most important of the carbohydrate constituents of kelp. Briefly described, algin may be separated from the seaweed in the following manner: The material is digested cold for 24 hours with a dilute solution of sodium carbonate or other alkali. A very thick, sirupy mixture results, which is filtered with suction. The filtrate is treated with a slight excess of sulphuric or hydrochloric acid. Immediately a heavy yellow precipitate is formed and floats in the watery liquid. In its water-holding power this body may well be compared with a sponge. On exposure to the air the color of the moist precipitate rapidly deepens to a dark brown, and on drying it shrinks to a dark-colored hard substance.

Stanford, in the investigations already mentioned (24–31) in this paper, concluded that algin prepared in this manner was a definite chemical body. He assumed that nitrogen was an essential constituent and even advanced a definite formula: $C_{76}H_{76}O_{22} \diagup^{NH_2.}_{\diagdown NH_2}$ Such a formula is without justification, since the elementary analysis was made on a highly contaminated sample. Stanford further described a series of salts of alginic acid with the heavy metals, and these, as well as the original algin, he believed to be of considerable commercial importance. Smith (21), in summarizing this and other work, compares algin with cottage cheese and quotes the following analysis made by Stanford: C, 44.39 per cent; H, 5.47 per cent; N, 3.77 per cent; O, 46.37 per cent.

More recently Kylin (15) has described an algin which he prepared from *Laminaria digitata* and from several related species. Many of the

characteristics correspond to those described by Stanford, although Kylin made his original extraction with water rather than alkali.

In this laboratory experiments were inaugurated to determine to what extent a similar substance might be present in the Pacific coast kelps, and in addition its general chemical characteristics. It was found that the maximum yield of algin was obtainable by digestion in the cold and with a dilute sodium-carbonate solution (2 per cent). The use of stronger alkalies or the application of heat is unfavorable, probably because of a tendency of the body to decompose under such conditions. The following procedure was adopted for the estimation of the algin fraction:

Two gm. of kelp were digested in the cold for 24 hours with a 2 per cent sodium-carbonate solution. The residue was filtered off and washed with cold water. Twenty c. c. of dilute hydrochloric acid were added to the filtrate, and the precipitate was allowed to stand for 24 hours. It was then filtered on a linen cloth, washed, dried, weighed, ignited, and the weight of the ash subtracted.

The percentages obtained by this method varied from 13 to 24, calculated on the dry kelp. (See Table III.) *Iridaea* spp. again form an exception, having only 1 per cent of this complex. The general properties of the algin thus obtained are as follows: Solubility in sodium carbonate, ammonia, and other alkalies, with formation of viscous solutions exceedingly difficult of filtration; insolubility in water, in strong acids, in alcohol, also in ether, benzine, turpentine, etc.; resistance to solution after complete drying; precipitation by copper and other heavy metals. Long standing in even a weak alkaline solution causes a decomposition or fermentation to take place, so that precipitation with acid is no longer possible.

A sample of algin was subjected to further purification by three reprecipitations with hydrochloric acid and two by alcohol from the slightly alkaline solution. The product was finally bleached with sulphurous acid, and was thoroughly washed and dried. The following analytical results were obtained:

	Per cent.
Nitrógen	0. 3
Ash	2. 2
Furfural calculated to pentosan	38. 6
Insoluble after treatment with concentrated nitric acid (cellulose derivative)	24. 5

Some of the moist precipitate was boiled for several hours with a 2 per cent solution of sulphuric acid. The solution gave a good reduction of Fehling's solution. Drying caused the substance to become very much more resistant to hydrolysis. Treatment with nitric acid did not give the mucic-acid test for galactan. We may conclude from these observations that algin is a very complex resistant compound (or mixture of com-

pounds) of the pentosan type, with cellulose possibly making up a part of the complex. Algin has weakly acid properties, forming soluble compounds with the alkali metals. Thus, the sodium alginate precipitated by alcohol is easily soluble in water, while the alginic acid is almost insoluble in water. To precipitate out the alginic acid requires apparently a definite concentration of the hydrogen ion. No precipitation occurs with the weakly dissociated organic acids unless present in excess. From an acetic-acid solution of algin, gelatinous precipitates may be obtained with a large number of metallic salts. Mr. L. L. Lieb, of this laboratory, has prepared a series of such compounds, as described in Table V.

TABLE V.—*Metallic alginates*

SOLUBLE ALGINATES (PRECIPITATED BY ALCOHOL)[a]

Metal.	Metallic salt used.	Color of fresh precipitate.	General properties of precipitate.
Li.........	Li (acetate).........	Silver white....	Gelatinous, transparent.
Na........	NaOH.............	White..........	"Stringy," brittle when dry.
Mg.......	Mg (acetate).........	.....do.........	Transparent, gelatinous.
NH$_4$......	NH$_4$OH............	Light yellow....	Light, gelatinous.
K.........	KOH..............	Transparent....	Light, fluffy.

INSOLUBLE ALGINATES (PRECIPITATED FROM ACETIC-ACID SOLUTION)

Al........	AlCl$_3$..............	White..........	Gelatinous, brittle when dry, brown color.
Ca........	CaCl$_2$.................	.....do.........	Gelatinous, glossy when dry.
Cr........	Cr(NO$_3$)$_3$...........	Light blue......	Heavy, nongelatinous.
Mn........	Mn(C$_2$H$_3$O$_2$)$_2$........	Light red......	Gelatinous, good gloss to paper when dry.
Fe″.......	FeSO$_4$.7H$_2$O.......	Light brown....	Gelatinous, brittle when dry.
Fe‴.......	FeCl$_3$..............	Brown..........	Gelatinous.
Co........	Co(NO$_3$)$_2$..........	Reddish.......	Gelatinous, good gloss to paper when dry.
Ni........	NiCl$_2$..............	Light green....	Gelatinous.
Cu........	CuSO$_4$..............	.....do.........	Do.
Zn........	ZnSO$_4$.............	Colorless.......	Gelatinous, silvery gloss to paper when dry.
Sr.........	Sr(NO$_3$)$_2$...........	Light brown...	Heavy gelatinous, transparent when dry.
Ag........	AgNO$_3$.............	White..........	Gelatinous, becomes dark red when dry.
Cd........	Cd(NO$_3$)$_2$..........	Colorless......	Gelatinous, becomes horny.
Sn″......	SnCl$_2$H$_2$O..........	White.........	Thick, gelatinous.
Sn⁗.....	SnCl$_4$.................	.....do.........	Do.
Ba........	BaCl$_2$.................	.....do.........	Gelatinous, good gloss to paper when dry.
Pt.[b].....	PtCl$_4$..............	Light brown....	Gelatinous.
Au.[b]......	AuCl$_3$.............	Red...........	Do.
Hg′........	Hg$_2$(NO$_3$)$_2$.........	White..........	Dense, white, gelatinous.
Pb........	Pb(C$_2$H$_3$O$_2$)$_2$........	Colorless.......	Gelatinous, like isinglass when dry.
Bi........	Bi(NO$_3$)$_2$..........	White.........	Gelatinous.
U.........	U(SO$_4$)$_2$.4H$_2$O......	Yellow........	Thick, gelatinous.

[a] All these soluble alginates give more or less gloss to paper.   [b] Precipitated from alcohol solution.

Stanford (30) prepared a considerable number of compounds similar to those described here, although his data presented some discrepancies. An inspection of the table will indicate certain theoretical possibilities for the use of alginates as sizes or mordants, but the practical difficulties of preparation would probably prevent any commercial application of such products in competition with the various low-priced materials now used for these purposes.

CARBOHYDRATES PRECIPITABLE BY ALCOHOL

The alcohol-precipitable matter was prepared by treating the dried and ground kelp with a weakly acid solution, and adding sufficient strong alcohol to the filtrate to cause a complete precipitation. The flocculent precipitate was filtered by suction and washed with alcohol; the weight of the ash was subtracted from the total dry weight. The product very readily absorbed water from the atmosphere, soon becoming mucilaginous. The dried substance contained 1.2 per cent of nitrogen and yielded furfural, corresponding to 13.2 per cent of pentosan. No color test was given with iodin and no reduction with Fehling's solution. The moist precipitate was boiled several hours with a 2 per cent solution of sulphuric acid when the solution produced considerable reduction of the alkaline copper solution. Upon drying, the precipitate became very resistant to solution and to hydrolysis. The percentages in the plant of the carbohydrate bodies precipitated by alcohol are much less than for algin. (See Table III.) In the plants of *Macrocystis pyrifera* the stems show uniformly higher percentages than the leaves.

CELLULOSE

A composite sample of fiber, obtained as in the crude fiber method, was treated by the method of Cross and Bevan (5, p. 94-95), by chlorination and boiling with alkaline sodium sulphite. Final decolorization was effected with potassium permanganate. Pure cellulose was thus obtained and was found to make up approximately one-half of the crude fiber, or calculated on the whole dry plant, 3 to 4 per cent.

HYDROLYSIS OF KELP

Acid hydrolysis of the dried kelp yielded copper-reducing substances only with great difficulty. Ten hours' boiling with a 2 per cent sulphuric-acid solution gave the following amounts of reduced copper calculated as dextrose:

	Per cent.		Per cent.
Macrocystis pyrifera, leaves	6. 4	*Nereocystis luetkeana*, stems	5. 7
Macrocystis pyrifera, stems	7. 0	*Egregia menziesii*	9. 0
Pelagophycus porra, leaves	9. 0	*Laminaria andersonii*	15. 7
Pelagophycus porra, stems	8. 6	*Iridaea* spp.	19. 8
Nereocystis luetkeana, leaves	5. 6		

FORMS OF SULPHUR IN KELP

Recent researches have emphasized the importance of sulphur as a constituent of plant tissues, and some investigators claim for this element an important rôle among the plant foods in which the soil may be deficient. Peterson (19) reviews the literature of the subject and gives tables of analyses showing the total content of sulphur and its forms in various plants considered to be exceptionally high in this constituent. Analyses of the kelp of California indicate much higher percentages of sulphur in many cases than those found in land plants of high sulphur content. It has been noted before that many marine algæ in other parts of the world contain considerable quantities of sulphur (6, Bd. 2, p. 820), but the proportion of sulphate to the total sulphur has not received attention. Table VI gives the percentages of total sulphur in various species of California kelps, and also the approximate division of the total sulphur content between organic and inorganic.

This was effected by leaching out all of the soluble sulphur and directly precipitating the solution with barium chlorid, using essentially the method of Folin. The sulphur precipitated directly by barium chlorid was subtracted from the total sulphur obtained by peroxid fusion and the difference regarded as organic sulphur. This was found to correspond approximately to the sulphur driven off on charring the sample.

TABLE VI.—*Percentage of sulphur in dried Pacific coast kelp*

No.	Material used.	Total sulphur.	Inorganic sulphur.	Organic sulphur.
1. 1	*Macrocystis pyrifera*, leaves	1. 25	0. 55	0. 70
1. 2	*Macrocystis pyrifera*, stems	. 82	. 38	. 44
2. 1	*Macrocystis pyrifera*, leaves	1. 14	. 46	. 68
2. 2	*Macrocystis pyrifera*, stems	. 72	. 36	. 36
15. 1	*Pelagophycus porra*, leaves	1. 03	. 49	. 54
15. 2	*Pelagophycus porra*, stems	. 71	. 28	. 43
29. 1	*Nereocystis luetkeana*, leaves	1. 27	. 82	. 45
29. 2	*Nereocystis luetkeana*, stems	. 45	. 14	. 31
42	*Egregia menziesii*	1. 17	. 32	. 85
43	*Laminaria andersonii*	2. 12	1. 07	1. 05
44	*Iridaea* spp.	8. 16	4. 52	3. 64

It will be noted that the leaves have a uniformly higher percentage of sulphur than the stems. For *Iridaea* spp. the amount of sulphur present is remarkably high. The exact nature of the organic combinations of sulphur is not known. Preliminary experiments indicate that the substances precipitated by alcohol contain a considerable proportion of organic sulphur; the protein sulphur may also be large. In order to test the presence of volatile sulphur compounds, steam distillations of kelp were made, in some cases from 10 per cent hydrochloric-acid

solutions, but no evidence of volatile sulphur could be obtained. Various samples of kelp were also boiled with alkali, but no tests for lead-blackening sulphur were apparent. The possible presence of volatile sulphur lost on drying has not yet been verified. Further work is planned to clear up these points.

FORMS OF IODIN IN KELP

Various researches have shown that many forms of marine life, such as the coral and the sponge, contain large amounts of iodin in combination with proteins or amino acids (9, 17, 33). By analogy it might be assumed that the 0.1 or 0.2 per cent of iodin present was also organically combined. Eschle (7) studied *Fucus vesiculosus* and *Laminaria digitata* with this in mind. He found that he could only extract 10 per cent of the iodin from the dried weed with boiling alcohol. From this and other extraction experiments he concluded that most of the iodin was organically combined.

Extractions of dried samples of Pacific coast kelps made in this laboratory indicate that nearly all the iodin is extractable by cold water or 90 per cent alcohol. From the aqueous solutions iodin may be set free by dilute potassium permanganate or potassium nitrite, which would lead to the inference that the iodin is present in ionic form. To determine whether the iodin could be completely extracted by water, a sample of dried and ground kelp was repeatedly digested and washed with warm water until the washings showed no further test for chlorin. The residue was found to still contain 5 per cent of the total quantity of iodin present. Another sample was treated in a similar manner, except that a very dilute alkali was used first for extraction. In this case the residue retained only a faint trace of iodin. It is possible that a small percentage of iodin is always present in organic combination, soluble in alkali, while a much larger amount exists as the iodid. Bromin is also found in the kelp, but only in one-fifth to one-tenth the quantity of iodin. The analysis of sea water shows a quite opposite condition, the amount of bromin largely exceeding that of iodin. There is a marked selective power in the kelp for iodin, although the exact function of this element is not known. Certainly the quantities of iodin retained by these plants are enormous as compared with the concentration in the sea water which bathes them. The selective action for potash is of course almost equally striking, but this difference is of interest; much of the potassium chlorid effloresces out as the plant dries, while no iodin is demonstrable in the effloresced salt. Many questions bearing on the essential or nonessential character of the various chemical elements present in kelp could be solved only by propagation in artificially controlled solutions. Such experiments would be of extreme interest, but would be difficult or impossible of execution.

ECONOMIC CONSIDERATIONS

FEEDING VALUE

The extensive use in Japan and Hawaii of certain seaweeds as articles of food has given rise to the suggestion that the giant kelp might be utilized for feeding man or animals. This question was discussed in a general way some years ago by Alsberg (4, p. 263–270), but he pointed out that practically no data regarding the composition of the kelps were available at that time. The chemical studies reported in the present paper make it evident that from the standpoint of nutrition the principal varieties of the California kelps could have but slight value. The carbohydrates are undoubtedly of a very resistant type, hydrolized with great difficulty, and their percentage utilization would necessarily be low. Saiki (20) has investigated this question for the carbohydrates of Irish moss (*Chondrus crispus*), several varieties of Japanese edible seaweeds, and agar-agar. Digestions were made with ptyalin, pancreatic amylase, and intestinal extract. In none of the cases was there any evidence of hydrolysis by the enzyms present. Feeding experiments on a human subject and on a dog gave very low coefficients of digestibility. This has been the import of many other experiments (16) in which pentosans, galactans, and similar carbohydrates have been investigated with reference to their nutritive value. It has never been shown that they are directly hydrolyzable by any of the enzyms of the digestive tract. Some value they unquestionably have because of bacterial decomposition, especially for animals of the ruminant type, but these resistant carbohydrates are at the best of low rank among feeding materials.

Analyses of the Pacific coast kelps show in some cases very appreciable percentages of nitrogen. If this were all in the form of utilizable proteins, it would make a very important addition to the feeding value, but it is doubtful whether such is the case. It has been shown earlier in this paper that a considerable portion of the nitrogen exists in the nonprotein form. Although the percentage of acid amid nitrogen is apparently very small, it would still be necessary to prove that the remainder of the soluble nitrogen was present in the form of suitably proportioned amino acids, before a high nutritive value could be assigned to the material. Furthermore, the nitrogenous compounds would undoubtedly be rendered less available because of the admixture of large percentages of highly resistant polysaccharids.

In order to recover the potash, it would be necessary to leach the kelp. Only the residue would ordinarily be considered for feeding purposes. Since much of the organic matter is soluble in water, the value of the residue would be still further decreased. Moreover, it is not believed that the kelp would produce a very palatable ration. Mr. F. W. Woll, of the University of California Division of Animal Industry, reports that cows

will not eat the leached or unleached fresh kelp unless it is well mixed with other feed.

In order to ascertain whether kelp might be preserved in the fresh state as a sort of silage, a sample of *Nereocystis luetkeana* was packed in an air-tight container and stored for three months. At the end of this period there was no indication of putrefaction. The acidity had increased slightly, the final percentage being 0.18 as lactic acid. The sample had become soft and "crumbly," but there was no formation of reducing substances or marked increase in soluble material.

UTILIZATION OF BY-PRODUCTS

Many uses were suggested by Stanford for the so-called algin. Various patents for the manufacture and application of this material were obtained (10–14). It was considered to be especially adapted for use in sizing papers and fabrics. That a substance of this nature might serve such a purpose is undoubtedly true, but that it would be commercially profitable is questionable. From a mechanical point of view the preparation of algin is difficult. The alkaline solution is extremely troublesome to filter, while the final product is very bulky, having only a very small proportion of dry matter. The dried material becomes very resistant to solvents. It would not be adapted to the preparation of spirit varnishes, since it is insoluble in alcohol, turpentine, and like solvents. It is true that an algin solution has a very high viscosity, but it does not follow that it possesses the properties of an adhesive, and such is, in fact, not the case.

Suggestions had been advanced that algin might serve for clarification of wines. Mr. W. V. Cruess, of the University of California Division of Enology, made several experiments to test this point. He found that the physical properties of the product did not well adapt it to the process of clarification. A further suggestion was to utilize the leached kelp in the manufacture of paper. It is difficult to understand how any of the usual types of paper could be prepared from a plant having such a low cellulose content. Redwood wastes and others of much greater possibilities are still to be utilized.

DESTRUCTIVE DISTILLATION

Balch and others have claimed that kelp might be destructively distilled and yield a profit. Balch (1) states that the volatile products from kelp, acetic acid, methyl alcohol, and tar "may be regarded as approximating in value those of beech wood." No experimental evidence is presented in support of this conclusion. In order to obtain data which would justify definite statements in regard to these points, distillation experiments were made in this laboratory. The apparatus used was an iron retort of about 1½ gallons' capacity, provided with a pyrometer and a suitable condenser. Distillation experiments made in this way

on a laboratory scale are open to the objection that they are not comparable with commercial practice. It was decided, therefore, to make control experiments under identical conditions, with materials of already ascertained value for purposes of destructive distillation. These distillations were made on oak sawdust and Douglas fir shavings. A large number of distillations were conducted under varying conditions, the results of which are recorded in Table VII.

TABLE VII.—*Comparative distillations of Pacific coast kelp, Douglas fir, and oak*

Run No.	Material used.	Quantity used.	Moisture.	Total distillate.	Total distillate less moisture.	Settled tar.	Charcoal.	Gas (by difference).	Total time of distillation.	Percentage of dry weight of material.		
										Acid as acetic.	Methyl alcohol (100 per cent).	Settled tar.
		Kg.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Hrs.			
a 3	Macrocystis pyrifera........	1	116	290	174	......	465	245	......	......	......	......
4	.....do.................	1	116	265	149	......	470	265	......	......	......	......
5	.....do.................	1	116	250	134	......	480	270	......	......	......	......
b 6	.....do.................	1	116	255	139	......	465	280	......	......	......	......
7	.....do.................	1	116	277	161	......	475	248	......	......	......	......
8	.....do.................	1	116	275	159	......	510	215	......	......	......	......
c 9	.....do.................	1	116	265	149	......	500	235	......	......	......	......
10	.....do.................	1	116	268	152	......	480	252	......	......	......	......
11	.....do.................	1	116	262	146	48	485	253	3	0.34	0.14	5.4
12	.....do.................	1	116	283	167	41	512	205	3¾	.49	(aver-	4.6
d 13	.....do.................	1	116	277	161	60	472	251	4	.28	age.	6.8
e 14	.....do.................	1	116	242	126	37	487	271	4	.28		4.2
f 15	.....do.................	1	116	217	101	30	515	268	8	......		3.4
23	.....do.................	1	116	277	161	60	485	238	4	.49	.20	6.8
g 24	.....do.................	1	116	305	189	55	460	235	8	.58	.18	6.2
16	Douglas fir (*Pseudotsuga taxifolia*).	1	190	414	224	60	260	326	3	1.5	.7	7.0
17	.....do.................	1	190	424	234	80	276	300	2½	......	.7	10.0
25	.....do.................	1	190	450	260	100	300	250	4	1.6	......	12.0
h 18	Oak (*Quercus* spp.) sawdust	1	366	550	184	45	200	250	4	2.9	1.1	7.0
19	.....do.................	1	313	424	111	60	230	346	3½	2.6	1.3	9.0
i 20	.....do.................	1	313	412	99	37	215	373	4½	2.5	1.2	6.0

a Retort kept at red heat 1½ hours.
b Heated rapidly to red heat.
c Very slow distillation at low heat, occupying 12 hours.
d Distillation started 1.20 p. m.; 1.45 p. m., pyrometer 120° C.; 2 p. m., 160° C.; 2.30 p. m., 200° C.; 3.10 p. m., 220° C.; 4.05 p. m., 240° C. (gases slightly combustible); 4.30 p. m., 360° C. (gas burns steadily); 4.50 p. m., 420° C.; 5.10 p. m., 500° C. (no more distillate).
e Distillation started 8.30 a. m.; 9.15 a. m., 160° C. (large watery distillate); 9.30 a. m., 200° C.; 10.35 a. m., 220° C.; 11.15 a. m., 280° C. (gases slightly combustible); 11.30 a. m., 310° C. (distillate oily; gas burns); 12.25 p. m., 500° C.
f Distillation started 9 a. m.; 9.30 a. m., 140° C.; 9.50 a. m., 170° C.; 10.45 a. m., 190° C.; 12 m., 200° C.; 2 p. m., 220° C.; 2.15 p. m., 290° C.; 3.20 p. m., 390° C.; 4.30 p. m., 400° C.
g Distillation started 8.35 a. m.; 9.10 a. m., 160° C.; 11 a. m., 230° C.; 12.45 p. m., 250° C.; 1 p. m., 300° C.; 2.45 p. m., 440° C.; 4.45 p. m., 530° C.
h Distillation started 8.35 a. m.; 9.05 a. m., 170° C. (gases burn); 9.15 a. m., 200° C.; 10.05 a. m., 270° C.; 11.50 a. m., 290° C.; 12.30 p. m., 530° C.
i Distillation started 1.05 p. m.; 1.40 p. m., 160° C.; 2.40 p. m., 200° C.; 3.20 p. m., 250° C.; 5.25 p. m., 500° C.

Inspection of this table shows that the distillates from the kelp, judged by their content of acetic acid and alcohol, had a value of only one-fifth to one-tenth that of the oak and fir distillates, a value so slight as to preclude any profitable recovery of the products. The yields for oak and fir approximate those obtained in larger experiments on similar materials, and it is very probable, therefore, that the general comparisons with kelp would hold even in distillations on a commercial scale.

The distillates obtained from the kelp were watery in appearance and had a very slightly acid reaction to litmus, although they contained considerable amounts of basic substances. By the Kjeldahl method 3.2 gm. of nitrogen was found in the total distillate from 1 kg. of dried kelp. The tar oils obtained with the distillate floated on the surface, having a specific gravity of 0.984. Their percentage varies from 4 to 7 on the basis of the dry kelp. The gases evolved from the kelp differed from those of the oak and fir in not being combustible during any of the earlier stages of the distillation. The charcoal residue in the retort was soft and of dull-gray color. Leaching experiments indicate that most of the potash may be recovered from the char as a high-grade product.

Further details of the above work will be considered in a later article.

SUMMARY

(1) After a brief résumé of the literature, the general chemical composition of the principal species of Pacific coast kelps is discussed. An extended series of analyses is presented, with experimental data concerning the nature of algin and other carbohydrate bodies present.

(2) The forms of nitrogen in the kelp are considered. Much of the nitrogen is found to be present in nonprotein form.

(3) Experiments are reported on the form of the iodin, of which only a small proportion is believed to be organically combined.

(4) The high content of organic sulphur in the kelp is noted and a table of analyses given.

(5) The economic phases are discussed with reference to feeding value and utilization of organic by-products. The results indicate only slight possibilities of commercial value in these directions.

(6) Comparative laboratory experiments on the destructive distillation of kelp are presented, and the conclusion is reached that kelp distillates are of no practical importance.

LITERATURE CITED

(1) Balch, D. M.
     1909. On the chemistry of certain algae of the Pacific coast. *In* Jour. Indus. and Engin. Chem., v. 1, no. 12, p. 777–787, illus.

(2) Bauer, R. W.
     1889. Ueber einer aus Laminariaschleim entstehende Zuckerart. *In* Ber. Deut. Chem. Gesell., Jahrg. 22, p. 618.

(3) Burd, J. S.
     1915. The economic value of Pacific coast kelps. Cal. Agr. Exp. Sta. Bul. 248, p. 183–215, 3 fig.

(4) Cameron, F. K., Moore, R. B., et al.
     1912. A preliminary report on the fertilizer resources of the United States. U. S. 62d Cong. 2d Sess. Senate Doc. 190, 290 p., 19 pl., maps.

(5) Cross, C. F., Bevan, E. J., and Beadle, Clayton.
     1903. Cellulose ... ed. 2, 328 p., 14 pl. New York.

(6) CZAPEK, Friedrich.
     1905. Biochemie der Pflanzen. 2 Bd. Jena.

(7) ESCHLE, ——.
     1897. Ueber den Jodgehalt einiger Algenarten. *In* Ztschr. Physiol. Chem., Bd. 23, Heft 1, p. 30–37.

(8) EULER-CHELPIN, H. K. A. S. von.
     1908. Grundlagen und Ergebnisse der Pflanzenchemic. T. 1, 238 p. Braunschweig.

(9) HENZE, M.
     1907. Zur Kenntnis der jodbindenden Gruppe der natürlich vorkommenden Jodeiweisskörper. *In* Ztschr. Physiol. Chem., Bd. 51, Heft 1/2, p. 64–70.

(10) KREFTING, A.
     1896. An improved method of treating seaweed to obtain valuable products [alginic acid, "tang acid"] therefrom. Eng. Pat. 11,538, May 27, 1896. (Abstract.) *In* Jour. Soc. Chem. Indus., v. 15, no. 10, p. 720.

(11) ——
     1898. An improved system or apparatus for treating sea-weed [alginic acid] for the manufacture of products therefrom. Eng. Pat. 12,416, June 2, 1898. (Abstract.) *In* Jour. Soc. Chem. Indus., v. 17, no. 9, p. 846.

(12) ——
     1898. Improvements in the manufacture of organic products from sea-weed. Eng. Pat. 13,151, June 11, 1898. (Abstract.) *In* Jour. Soc. Chem. Indus., v. 17, no. 9, p. 846.

(13) ——
     1898. Improvements in the manufacture of organic products from sea-weed. Eng. Pat. 13,289, June 14, 1898. (Abstract.) *In* Jour. Soc. Chem. Indus., v. 17, no. 9, p. 846.

(14) ——
     1900. Useful products from sea-weed. Eng. Pat. 8,042, April 17, 1899. (Abstract.) *In* Jour. Soc. Chem. Indus., v. 19, no. 4, p. 361.

(15) KYLIN, Harald.
     1913. Zur Biochemie der Meeresalgen. *In* Ztschr. Physiol. Chem., Bd. 83, Heft 3, p. 171–197.

(16) McCOLLUM, E. V., and BRANNON, W. A.
     1909. The disappearance of pentosans from the digestive tract of the cow. *In* Jour. Amer. Chem. Soc., v. 31, no. 11, p. 1252–1260.

(17) MENDEL, L. B.
     1900. On the occurrence of iodine in corals. *In* Amer. Jour. Physiol., v. 4, no. 5, p. 243–246.

(18) MÜTHER, A., and TOLLENS, B.
     1904. Ueber die Producte der Hydrolyse von Seetang (Fucus), Laminaria and Carragheen-Moos. *In* Ber. Deut. Chem. Gesell., Jahrg. 37, No. 2, p. 298–305.

(19) PETERSON, W. H.
     1914. Forms of sulfur in plant materials and their variation with the soil supply. *In* Jour. Amer. Chem. Soc., v. 36, no. 6, p. 1290–1300.

(20) SAIKI, T.
     1906. The digestibility and utilization of some polysaccharide carbohydrates derived from lichens and marine algæ. *In* Jour. Biol. Chem., v. 2, no. 3, p. 251–265.

(21) SMITH, H. M.
    1905. The utilization of seaweeds in the United States. *In* U. S. Bur. Fisheries
        Bul., v. 24, 1904, p. 169–181, illus., pl. 5.
(22) ———
    1905. The seaweed industries of Japan. *In* U. S. Bur. Fisheries Bul., v. 24,
        1904, p. 135–165, illus., pl. 1–4.
(23) SMITH, Watson.
    1885. E. C. C. Stanford's new method of treating seaweed. *In* Jour. Soc.
        Chem. Indus., v. 4, no. 9, p. 518–520.
(24) STANFORD, E. C. C.
    1862. On the manufacture of kelp. (Abstract.) *In* Chem. News, v. 5, no. 120,
        p. 167.
(25) ———
    1876. Distillation of seaweed. *In* Chem. News, v. 34, no. 888, p. 237.
(26) ———
    1877. On the manufacture of iodine. *In* Chem. News, v. 35, no. 909, p. 172–175.
(27) ———
    1883. Iodine in cod liver oil. *In* Chem. News, v. 48, no. 1251, p. 233–234.
(28) ———
    1883. On algin: a new substance obtained from some of the commoner species
        of marine algæ. *In* Chem. News, v. 47, no. 1227, p. 254–257; no. 1228,
        p. 267–269.
(29) ———
    1884. On algin. *In* Jour. Soc. Chem. Indus., v. 3, no. 5, p. 297–301. Dis-
        cussion, p. 301–303.
(30) ———
    1886. On alginic acid and its compounds. *In* Jour. Soc. Chem. Indus., v. 5,
        no. 4, p. 218–221.
(31) ———
    1899. Improved pharmaceutical preparations of soluble and insoluble alginates
        of metallic and other bases. Eng. Pat. 8075, Feb. 18, 1899. (Abstract.)
        In Jour. Soc. Chem. Indus., v. 18, no. 4, p. 398.
(32) STEWART, G. R.
    1915. Studies on the availability of the nitrogen in Pacific coast kelps. *In*
        Jour. Agr. Research, v. 4, no. 1, p. 21–38.
(33) WHEELER, H. L., and MENDEL, L. B.
    1909. The iodine complex in sponges (3, 5-diiodtyrosine). *In* Jour. Biol.
        Chem., v. 7, no. 1, p. 1–9.

SOURCES OF THE EARLY INFECTIONS OF APPLE BITTER-ROT [1]

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INTRODUCTION

Notwithstanding the excellent work which has been done by previous investigators, the problem of determining the sources from which the early infections of the apple bitter-rot fungus (*Glomerella cingulata*) may arise has never been worked out with completeness sufficient to account for the heavy initial damage sometimes caused by this disease. Often bitter-rot will break out suddenly, and every apple (*Malus* spp.) in an orchard will be affected in an incredibly short time. In the Ozark region of Arkansas during the season of 1914 the crops of six of the orchards under the writer's observation were within two weeks almost entirely ruined by bitter-rot. Nearly all the apples on these trees were infected comparatively early in the season—i. e., about July 1—a large majority of them having from 50 to 100 points of infection. The suddenness of the appearance of the disease and the almost simultaneous infection of the fruit over the whole orchard strongly indicated that practically all the rotten spots were caused by spores which had washed down from primary sources of infection. In one orchard soon after the disease broke out nearly every apple was found to be affected with the small blister-like spots characteristic of the early stages of the disease (Pl. VII, fig. 1). These spots had not yet developed far enough to produce acervuli and were evidently due to infection by spores from over-wintering or primary sources.

The later infections are, of course, easily accounted for because the fungus forms acervuli in the rot areas of the earlier infections and from these the spores may be washed by rain or carried by insects to sound apples, which, if conditions are favorable, may become diseased and in turn become sources of infection. Thus, we may have primary sources of infection, which may continue to act as such throughout the season, and secondary sources of infection, consisting of the diseased fruits of the current season.

The primary sources of infection therefore become of great importance in the control of the disease, especially when they are present in great

abundance.   Under ordinary conditions, even with the weather favor-
able to the development of the fungus, bitter-rot may be practically
prevented by spraying with Bordeaux mixture.   When, however, in
addition to favorable weather conditions, the primary sources of infec-
tion are as abundant as they are in some of the orchards of the Ozarks
and probably other sections in which the disease is prevalent, spraying
alone will, by reducing the number of infections, only retard rather than
prevent.   To gain success by spraying it would be necessary to keep
the entire surface of every apple continually covered with Bordeaux
mixture, a physical impossibility.

HISTORICAL REVIEW OF LITERATURE

The early infections of bitter-rot have been explained somewhat differ-
ently by different writers, all of whom doubtless give correct explana-
tions for the particular regions or orchards in which their investigations
were made.   None of these writers, however, has made his investigations
complete enough to account for the numerous early infections which
sometimes occur.

Simpson, of Illinois, discovered that primary infections of bitter-rot
were associated with a certain type of twig canker (Burrill and Blair,
1902, p. 355; Von Schrenk and Spaulding, 1903, pp. 30–31).[1]

Burrill and Blair (1902, p. 356) discuss the fungus in relation to early
infections as follows:

> It therefore became evident that the disease on apples could come from these spots
> on the branches, and everything now goes to show that except in the few cases that the
> rot mummies hang over on the trees, the first or early infection comes solely from these
> limb cankers.   *   *   *   It now seems to be commonly true that the cankers are few
> in number, at least upon the kinds of trees ordinarily planted in Illinois and not over
> 15 years of age.

Clinton (1902) expresses the belief that the early infections come from
the ascogenous stage of the fungus, developing in mummies of the pre-
ceding year.

Hasselbring found that in mummied apples kept out of doors the fungus
ordinarily retains its vitality in a dormant state in the winter and in May
or later under proper conditions again begins to produce conidia (Burrill
and Blair, 1902, p. 354).

Von Schrenk and Spaulding (1903, pp. 37–38) showed by inoculations
that the limb cankers discovered by Simpson were actually caused by the
bitter-rot fungus.   They also state:

> The apparently erratic behavior of the bitter rot can be explained in part since the
> discovery of the canker stage of the fungus.   After its introduction into an orchard
> or on one tree the fungus attacks one or more branches, probably early in the summer,
> and produces a canker.   The next year the spores from this canker will be washed
> down on the ripening fruit by a rain.   The water is sprayed from the branch on which
> the canker is situated to the lower branches in the form of a cone, and one or more
> spores will probably fall on every apple within such a cone.   The presence of the

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[1] Bibliographic citations in parentheses refer to "Literature cited," p. 64.

winter stage of the fungus will explain why the rot is apt to recur on the trees affected the year before with the bitter rot, and also why the disease should first appear on such trees. The cankers produce spores early in the season, and from the trees which have cankers the disease spreads to neighboring trees. * * * It now seems probable that the mummies play a comparatively small part in serving as distributing points for spores from year to year.

Alwood (1902, pp. 264–265, 270), after extensive investigations of the disease in Virginia, states:

Diligent search of the limbs failed to show any bitter-rot cankers on these [susceptible] varieties mentioned. * * * In no instance have we been able to find the presence of the bitter-rot fungus on the limbs or trunks of apple or pear, though we have especially watched for its occurrence since the appearance of the publication cited [Burrill and Blair]. * * * It appears to be well established that the mummied fruits hanging to the trees and the rotted fruits upon the soil constitute in a large measure the source of the annually recurring infection.

Scott (1906, p. 12), after investigating the disease in Virginia, agrees with Alwood in that he considers mummies as the chief sources of infection. He states:

The results lead to the conclusion that the overwintering mummies hanging on the trees constitute the chief source of infection, at least in this particular region. In the majority of cases examined a mummy could be found in the upper portion of the infected area, but in no case was there found associated with such outbreaks any cankers that could be identified as bitter-rot cankers.

Shear and Wood (1913, p. 76) obtained *Glomerella cingulata* from a great variety of plants, and it is possible that in some cases early infections may come from hosts other than the apple.

INVESTIGATIONS IN THE OZARKS

The large number of primary infections in some of the orchards of the Ozarks from which mummies had been practically all removed and in which the bitter-rot cankers as described by previous writers were few or wanting led the writer to undertake to discover from what sources the early infections were arising in such serious abundance. It was impossible to believe that mummies and bitter-rot cankers, so few in themselves, could be the sole harboring places of a fungus which could cause from 10 to 200 rotten spots to appear on nearly every apple on large, heavily laden trees.

In this region cankers and dead areas on limbs, due to various causes, are very abundant. The Illinois apple-tree canker, caused by *Nummularia discreta*, is a very prevalent and serious disease. Cankers and dead areas due to *Bacillus amylovorus*, *Phyllosticta solitaria*, and various physiological and mechanical causes are also quite numerous. In some of the orchards it is almost impossible to find a branch or twig which does not show several of the cankers caused by *P. solitaria*.

Considering these cankers as possible sources of early infections, all cankers and dead wood, in so far as practicable, were removed in the

spring of 1914 from parts of two orchards in which the disease in pre-
vious years had not proved amenable to spraying. In one of these
orchards the part from which the cankers had been eradicated was the
section of the orchard in which during previous years bitter-rot had been
most destructive. The fruit of this part came through the season prac-
tically free from rot, while about 50 per cent of the fruit of the part from
which the cankers were not removed was destroyed by the disease.
Both of these parts were sprayed four times at intervals of two weeks,
beginning June 15. In the second orchard the fruit of neither plot was
sprayed, and all of it eventually rotted. In the plot in which the
cankers were allowed to remain every apple was infected by the middle
of July, whereas in the plot from which the cankers and dead wood had
been removed destruction was not complete until two months later.
Every apple in the untreated plot was evidently infected from primary
sources, since there were as yet no secondary sources. While an occa-
sional apple was found which showed only 1 infection, nearly every one
of them showed at least 50 and many of them were literally covered
with the tiny, blister-like spots. In the treated plot early infections
were considerably less in number, and a majority of the fruit was free
from them. Later in the season, however, all fruit that had escaped the
early infections finally became infected from secondary sources.

On May 15 a cankered limb from the second orchard was brought into
the laboratory and kept in a moist chamber for 24 hours. This canker
resembled in every way the limb cankers as described and figured by
Burrill and Blair and Von Schrenk and Spaulding (Pl. VII, fig. 2). It
was a black, sunken, oval area, with many slight rifts or cracks in the
bark through which, after the limb had remained in a moist chamber for
24 hours, an abundance of the characteristic pink acervuli appeared.
Near the center of this canker was a small dead spur through which
infection probably took place.

Cankers resembling in every way published descriptions and figures of
bitter-rot cankers were also collected on June 3 and many times thereafter.

During the month of May there was collected from one of these orchards
a limb which was badly infected with *Nummularia discreta*, and while
spore masses of *Glomerella cingulata* were not abundant on it, yet enough
were present to make it a dangerous source of infection under proper
conditions. Later in the season spores were many times obtained from
Nummularia cankers.

Spore masses of the fungus were also found on a long dead, well-
delimited part of a large limb in one of the orchards before mentioned.
This area was about 8 cm. wide and about 2 meters long. Wide cracks
along its margins sharply separated it from the living part of the limb
(Pl. VII, fig. 3). Such strips of dead tissue are usually assigned to
injury by freezing or to the death of roots on one side of the tree. While

the spores from this source were comparatively few, they were, nevertheless, sufficient to give the disease a good start. In the same orchard an occasional fruit spur was found from which spores were somewhat sparingly produced after it had been kept in a moist chamber for from 24 to 48 hours. The part from which the fungus was obtained was the dead tip on which the fruit of the preceding year had been borne.

In at least two well-authenticated cases acervuli were found on the injured parts of small limbs which had been nearly girdled by the organism of pear-blight (*Bacillus amylovorus*). These cankers had been caused by the blight organism infecting and killing a small twig and going thence into the tissues of the limb at the base of the twig (Pl. VII, fig. 4).

In one orchard which had been badly infected for years it was possible to find the fungus on almost any sort of cankered or injured limb. From directly above a mass of badly infected apples still hanging on the tree, a small limb having a long dead area just beginning to be healed over through the formation of callus was removed (Pl. VII, fig. 5). This injury had apparently been brought about by mechanical means; probably the limb had been severely scraped by the tower of a power sprayer or by a wagon box or hayrack, all of which were accustomed to pass through the orchard at frequent intervals. After a short time in a moist chamber acervuli appeared from beneath narrow strips of dead bark which lay near the angle formed by the dead area and the overlapping callus. This injured area was 19 cm. long and 4 mm. wide and contained innumerable acervuli. That this mechanically injured limb had served as a source of direct infection was indicated by the fact that there were a large number of badly infected fruits just below it, whereas all the apples above it were sound. No other possible sources of infection were present.

This orchard was also badly infected by the apple-blotch fungus (*Phyllosticta solitaria*). The cankers caused by this organism were quite numerous on the smaller limbs and branches, especially in the older part of the orchard which had been practically abandoned. In connection with some work with the apple-blotch fungus the writer had occasion to scrape from these cankers (Pl. VII, fig. 6) spore-bearing pycnidia of *Phyllosticta solitaria*, which, after being crushed, were placed in Van Tieghem cells so that spore germination might be observed. In many cases bits of bark were accidentally carried into the Van Tieghem cells along with the pycnidia and spores of the blotch fungus. Repeatedly during the month of May and later as well there grew out from these small bits of bark hypæ which produced spores (conidia) of the bitter-rot fungus. Cultures from these spores produced the characteristic acervuli and conidia and often the perithecia and ascospores of *Glomerella cingulata*.

Masses of spores were also obtained many times from mummies; and where mummies are present, they undoubtedly are important sources

of infection. In many of the badly infected orchards, however, they had been removed both from the trees and from the ground.

The fungus from all the sources mentioned was positively identified as *Glomerella cingulata* not only by microscopical observations and spore measurements but by spore germination and growth on artificial media as compared with germination and growth from spores from actual cases of bitter-rot of the fruit. Also in all cases sound, sterile apples were inoculated with spores from pure cultures, the typical bitter-rot disease was brought about, and the fungus reisolated. Thus, the spores from every source of infection discussed as such in this paper were proved to be those of the bitter-rot fungus.

SUMMARY

(1) Previous writers have shown that the apple bitter-rot fungus (*Glomerella cingulata*) may pass the winter in mummied apples of the preceding year and in bitter-rot cankers from which the early infections of the following season may come. Other plants also may be possible sources of infection.

(2) The writer has shown that in apple orchards where the infections have been severe the fungus may winter over on almost any cankered or dead parts of the tree, including the Illinois apple-tree canker due to *Nummularia discreta;* dead tips of fruit spurs; dead parts of limbs due to injury by freezing or to death of roots; branches injured by mechanical means; cankers caused by the pear-blight organism (*Bacillus amylovorus*); twig cankers caused by the apple-blotch fungus (*Phyllosticta solitaria*).

(3) Eradication of cankers greatly reduced the number of early infections of the disease, though removal of all small dead parts, such as dead tips of fruit spurs and small mechanically injured places, is, of course, practically impossible.

LITERATURE CITED

ALWOOD, W. B.
  1902. Orchard studies. XV. The bitter rot of apples. Va. Agr. Exp. Sta. Bul.
    142, p. 252–279, illus.
BURRILL, T. J., and BLAIR, J. C.
  1902. Bitter rot of apples. Ill. Agr. Exp. Sta. Bul. 77, p. 351–366, illus.
CLINTON, G. P.
  1902. Apple rots in Illinois. Ill. Agr. Exp. Sta. Bul. 69, p. 189–224, illus.
SCHRENK, Hermann von, and SPAULDING, Perley.
  1903. The bitter rot of apples. U. S. Dept. Agr. Bur. Plant Indus. Bul. 44, 54 p.,
    9 fig., 9 pl.
SCOTT, W. M.
  1906. The control of apple bitter-rot. U. S. Dept. Agr. Bur. Plant Indus. Bul.
    93, 33 p., 1 fig., 8 pl.
SHEAR, C. L., and WOOD, Anna K.
  1913. Studies of fungus parasites belonging to the genus Glomerella. U. S. Dept.
    Agr. Bur. Plant Indus. Bul. 252, 110 p., 4 fig., 18 pl.

PLATE VII

Fig. 1.—Givens apple having numerous small blister-like infections of bitter-rot.

Fig. 2.—Bitter-rot canker from a Jonathan apple tree.

Fig. 3.—Part of a branch of a Givens apple tree which had been injured probably by freezing. Acervuli of the bitter-rot fungus were obtained from the dead part of this branch.

Fig. 4.—Apple branch showing blighted area on which acervuli of the bitter-rot fungus were found.

Fig. 5.—Mechanically injured branch of a Missouri Pippin apple tree. Acervuli of the bitter-rot fungus were found about the margins of the injured part.

Fig. 6.—Branch of Missouri Pippin apple tree affected with apple-blotch. The bitter-rot fungus was found to be wintering over in blotch cankers.
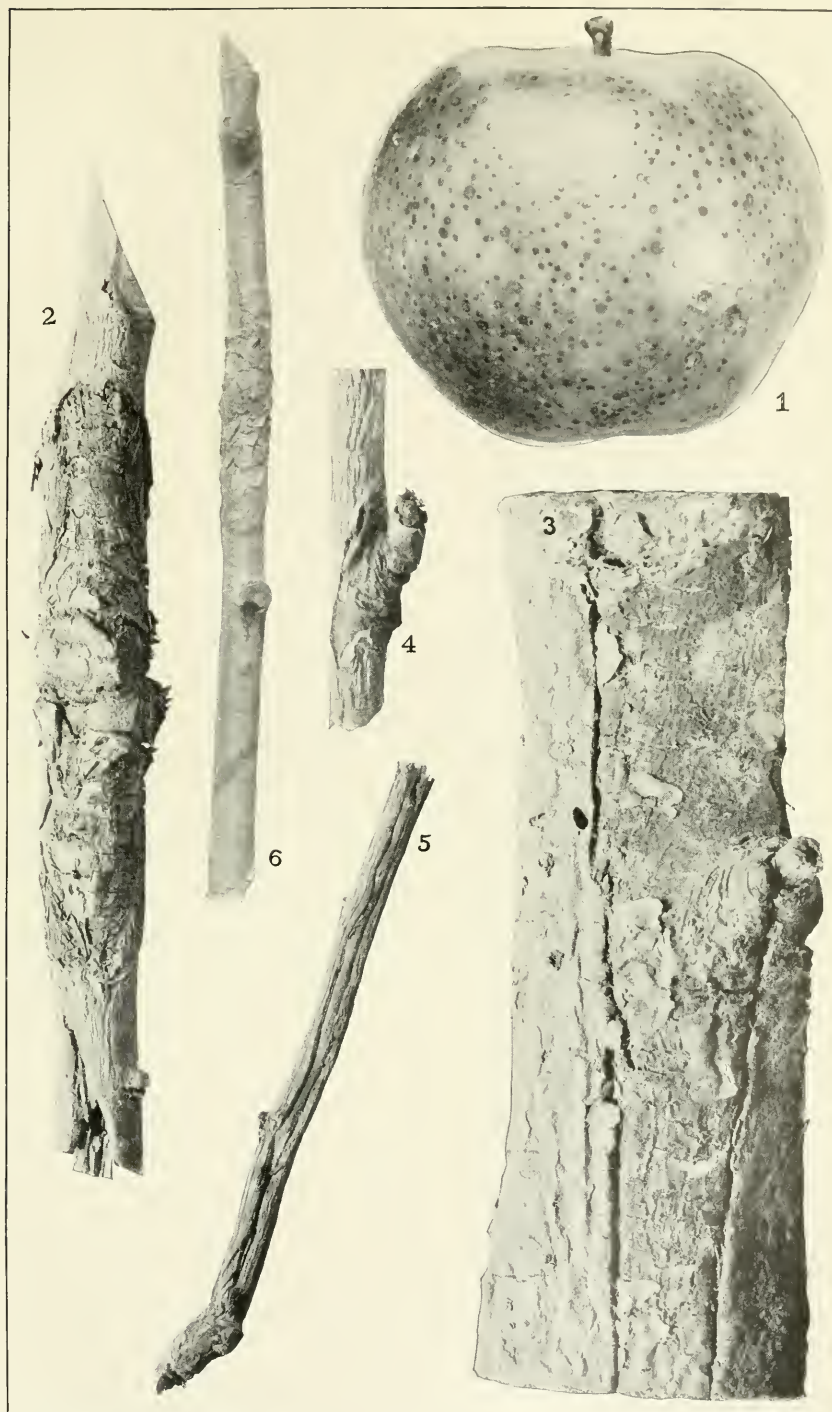
A BACTERIOLOGICAL STUDY OF METHODS FOR THE DISINFECTION OF HIDES INFECTED WITH ANTHRAX SPORES

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INTRODUCTION

The number of hides and skins imported into this country each year amounts to many millions. Since these come to us from all quarters of the globe, it is evident that there is danger that they will bring with them infectious material which may cause disease among animals and human beings.

On account of the great resisting power of the anthrax spore, hides and skins imported from countries where anthrax is prevalent are regarded as especially dangerous; and inasmuch as methods of disinfection which will destroy the anthrax spore may be expected to kill other organisms with ease, considerable attention has been devoted to the problem of securing a disinfectant that will destroy the anthrax spores without damaging the hides and skins. Among the numerous processes which have been suggested, that devised in 1910 by Seymour-Jones (16)[2] has attracted much attention, while more recently the Schattenfroh (12) method has been declared by various investigators to be equally efficient and by some even more so.

As Eurich (1, 2), Ponder (9, 10), Seymour-Jones (16), and others have pointed out, the spores of anthrax are found chiefly in connection with blood stains, and as these, together with other material with which the spores are likely to be associated, are colloidal in nature, the problem, as Seymour-Jones expresses it, is to get at the anthrax spore "when imbedded in a gelatinous, albuminous, or other colloidal body without injury to the material or fabric to be disinfected."

OUTLINE OF SEYMOUR-JONES AND SCHATTENFROH METHODS OF DISINFECTION

Seymour-Jones (16) proposes to attain the desired result by the use of mercuric chlorid and formic acid. He holds that the acid causes the hide and the various associated colloidal substances to swell, absorb water, and become soft and tender, thus furnishing favorable conditions for the action of the mercuric chlorid. Under these conditions he con-

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siders a dilute solution of mercuric chlorid sufficient for disinfection. After disinfection hides are transferred to a saturated solution of common salt, whereby, it is claimed, they will be shrunk and brought to the "wet salted" state. The dilutions recommended are mercuric chlorid, 1 part in 5,000, with 1 per cent of formic acid; and the time of exposure to the disinfectant, 24 hours.

One of the first workers to investigate the Seymour-Jones process was C. W. Ponder (9, 10). He found that artificially infected pieces of hide were not disinfected in 24 hours by a solution of mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, in 4 cases out of 10 and concluded that the effective dilution of mercuric chlorid lay between 1 to 1,000 and 1 to 5,000. In spite of these results he recommends the service use of mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, on the ground that his tests were made more rigorous than was necessary to meet the conditions obtaining in actual routine disinfection. It is worthy of note that he made no attempt to neutralize the disinfectant before testing the results by cultures and by inoculation of animals. Moegle (7) and Schnürer (13) have also reported favorable results with the Seymour-Jones method.

The investigations of Ševčík (14) controvert all these favorable results. By neutralizing the disinfectant with sodium sulphid he was able to obtain living and virulent anthrax bacilli from spores treated with very strong dilutions of mercuric chlorid and formic acid, even when the time of exposure was extended to a number of days. Judging from his published results, it would require a dilution of mercuric chlorid, 1 to 500, plus 1 per cent of formic acid, to destroy anthrax spores in 24 hours. The use of sodium sulphid in this manner does not seem unreasonable, since, as a matter of fact, many tanners use this substance for dehairing hides. Hilgermann and Marmann (4) have obtained similar results with the Seymour-Jones method, using ammonium sulphid as a neutralizing agent.

Another method for the disinfection of hides which has recently come into prominence is the method of Prof. Schattenfroh (12), which depends upon the use of hydrochloric acid and sodium chlorid. The amounts recommended for use at room temperature are 2 per cent of the acid and 10 per cent of the salt, with a 48-hour exposure. At higher temperatures weaker dilutions may be employed.

Gegenbauer and Reichel (3) have carried on an extensive research with this method and report entirely favorable results. They state that they consider the Seymour-Jones method inefficient on account of the low concentration of mercuric chlorid and also object to its use because of the discoloration by mercuric sulphid when sodium sulphid is used for dehairing. Their statements regarding the Seymour-Jones method appear to be based upon experimental work not yet published. The favorable results obtained by Gegenbauer and Reichel with the Schatten-froh method are confirmed by the favorable results obtained by Hilger-

mann and Marmann (4) as the result of comparative experiments with the Seymour-Jones and Schattenfroh methods.

Ševčík's comparison (14) of the two methods is interesting, but is not fair to the Schattenfroh method, as he admits, because of the use of solutions based on the percentage of "hydrochloric acid" rather than on the percentage of hydrochloric-acid gas.

EXPERIMENTAL WORK ON GERMICIDAL EFFICIENCY OF DISINFECTANTS

The experimental work was undertaken primarily with a view to determining the value of the Seymour-Jones method, and for that reason this paper deals largely with work done with that method, although some attention was paid to others, especially the Schattenfroh method.

In the absence of a supply of naturally infected hides it was necessary to make the experiments upon pure cultures and artificially infected pieces of hide. Although Ševčík (14) states that naturally infected hides are better for test preparations than those artificially infected, it does not seem that the difference is as great as he claims. Certainly his results with naturally infected hides, where the disinfectant was not neutralized, correspond very closely to results obtained by Ponder (9, 10) with artificially infected hides.

EXPERIMENTAL PROCEDURE

For preliminary work the Hill "rod" method (5) seemed best adapted; so this was used, with some modifications. The method as modified is as follows: Glass rods three-sixteenths of an inch in diameter and 8 inches long are etched at one end, the etched portion being about 1 inch long. Cotton is wrapped about the rods near the end not etched and the rods thrust into test tubes so as to engage the cotton in the mouth of the tube. The tubes containing the rods are sterilized by dry heat (150° C.) for one hour or more. In making tests the rods are removed and the etched portion dipped into a suspension made from a culture of the organism employed and this allowed to dry on the rod.

Rods so infected are transferred to test tubes containing the disinfectant to be tested and there exposed to its action for varying lengths of time. After exposure the rods are washed with sterile water in order to remove traces of the disinfectant and are then transferred to tubes containing bouillon or agar, which are incubated for at least 48 hours at 37.5° C. The suspension used in infecting the rods is made from the surface growth on an agar tube by rubbing up in several cubic centimeters of sterile water enough of the growth to give a suspension of approximately the same density as a 24-hour bouillon culture of *Bacillus typhosus*. For a non-spore-bearing organism the culture should be 24 hours old, while for spore-bearing organisms cultures 1 to 2 weeks old are usually the most suitable.

In making tests with disinfectants containing mercury it is advisable to dip the rods into a saturated solution of hydrogen sulphid or an aqueous solution of some sulphid before placing them in subculture tubes. In this connection it should be mentioned that media of acid reaction have been found to exert an inhibitory action upon the growth of *Bacillus anthracis* after exposure to disinfectants. For that reason the media employed in these experiments have been neutral or slightly alkaline.

A considerable number of tests by the rod method were made with organic matter added to the disinfectant. This was done by removing a certain portion of the total volume of disinfectant and substituting a like amount of defibrinated blood.

Inasmuch as the use of a solution of sodium chlorid did not seem essential in experiments upon "naked" anthrax spores, since this salt is said by Seymour-Jones to be used in his method to reduce the swelling of the hides caused by formic acid, a common salt solution was not used in the "rod" method experiments.

MERCURIC CHLORID AND FORMIC ACID

I. EXPERIMENTS BY ROD METHOD, USING BUREAU OF ANIMAL INDUSTRY STRAIN OF BACILLUS ANTHRACIS

These experiments were designed to show the germicidal efficiency of mercuric chlorid ($HgCl_2$) with and without formic acid (CH_2O_2) and with and without the addition of defibrinated blood.

In experiment 1 (Table I) the rods were infected by using an agar culture 2 weeks old for making the spore suspension. Microscopical examination of the suspension showed that plenty of spores were present. Each rod was exposed to 5 c. c. of disinfectant for 24 hours and was then washed in 20 c. c. of hydrogen-sulphid solution or sterile distilled water.

The rods were then transferred to subculture tubes of exactly neutral broth and incubated at 37.5° C. for three days.

TABLE I.—*Germicidal efficiency of mercuric chlorid, with and without formic acid, and of phenol by the rod method, without addition of organic matter* [a]

EXPERIMENT I

Rod No.	Disinfectant (5 c. c.) and dilution.	Time of exposure.	Results after incubation for—		
			18 hours.	1 day.	3 days.
		Hours.			
1	Mercuric chlorid (1:5,000)	24	No growth	Growth	Strong growth.
2	Mercuric chlorid (1:5,000)+formic acid (1 per cent).	24	.....do	No growth	No growth.
3	Control rod	(b)	Strong growth	Strong growth	Strong growth.
4	Mercuric chlorid (1:5,000)	24	No growth	No growth	No growth.
5	Mercuric chlorid (1:5,000)+formic acid (1 per cent).	24	.....do	.....do	Do.
6	Control rod	(b)	Strong growth	Strong growth	Strong growth.
7	Phenol (5 per cent)	24	No growth	Growth	Do.
8	Do	48	.....do	.....do	Do.

[a] Rods 1, 2, and 3 washed with hydrogen-sulphid solution, and Nos. 4, 5, 6, 7, and 8 with sterile water.
[b] Not exposed.

The results of the above experiment indicate that mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, is efficient where mercuric chlorid alone is not and that the hydrogen-sulphid solution should be used to neutralize the disinfectant before putting the rods into subculture tubes. The result after 48 hours' exposure to 5 per cent of phenol indicates the resisting power of the anthrax spores. The next experiment consisted of short exposures with the addition of defibrinated blood. This experiment was intended to test the efficiency of the method of disinfecting hides prescribed in Circular No. 23 of the Treasury Department, which consisted in immersion of hides for half an hour in a solution of mercuric chlorid, 1 to 1,000.

The technique was similar to that described for experiment 1, except that all rods were washed with hydrogen-sulphid solution and defibrinated blood was added so as to make up 10 per cent of the volume of the disinfectant in each tube. The results are given in Table II, experiment 2.

Experiment 2 indicates that anthrax spores are not destroyed in the presence of defibrinated blood by mercuric chlorid, 1 to 1,000, without formic acid, or by mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, even with an exposure of two hours.

An experiment with stronger dilutions of mercuric chlorid plus formic acid was now tried. Defibrinated blood was added in the proportion of 10 per cent of the total volume. (See Table II, experiment 3.)

TABLE II.—*Germicidal efficiency of mercuric chlorid, with or without formic acid, by the rod method, with the addition of defibrinated blood* [a]

EXPERIMENT 2

Rod No.	Disinfectant (5 c. c.) and dilution.	Time of exposure.	Result.
		Hours.	
1	Mercuric chlorid (1:1,000)..	1/4	Growth.
2	Do..	1/2	Do.
3	Do..	1	Do.
4	Do..	2	Do.
5	Mercuric chlorid (1:5,000)+formic acid (1 per cent).............	1/4	Do.
6	Do..	1/2	Do.
7	Do..	1	Do.
8	Do..	2	Do.
9	Control rod...	(b)	Do.

EXPERIMENT 3

1	Mercuric chlorid (1:1,000)+formic acid (1 per cent).............	1/4	No growth.
2	Do..	1/2	Do.
3	Do..	1	Do.
4	Do..	2	Do.
5	Mercuric chlorid (1:2,000)+formic acid (1 per cent).............	1/4	Do.
6	Do..	1/2	Do.
7	Do..	1	Do.
8	Do..	2	Do.
9	Control rod...	(b)	Growth.

EXPERIMENT 4

1	Mercuric chlorid (1:1,000)..	24	No growth.
2	Mercuric chlorid (1:5,000)+formic acid (1 per cent).............	24	Do.
3	Control rod...	(b)	Growth.

[a] Subculture tubes incubated one week. Rods washed with hydrogen-sulphid solution.
[b] Not exposed.

Returning to conditions more closely resembling the Seymour-Jones method, experiment 4 was carried out with a 24-hour exposure to the disinfectant plus 10 per cent of defibrinated blood. The results are given in Table II, experiment 4.

The results of the preceding experiments indicated that in the presence of 10 per cent of defibrinated blood anthrax spores are not destroyed in 2 hours by mercuric chlorid, 1 to 1,000, without formic acid, nor by mercuric chlorid, 1 to 5,000, with 1 per cent of formic acid, but that they are destroyed by mercuric chlorid, 1 to 2,000, with 1 per cent of formic acid, under the same conditions. On the other hand, anthrax spores are destroyed by mercuric chlorid, 1 to 1,000, without formic acid, and by mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, even in the presence of defibrinated blood, when the time of exposure is 24 hours.

On account of the greatly increased germicidal power of mercuric chlorid in the presence of formic acid observed in the foregoing preliminary experiments, it was deemed advisable to test the germicidal power of mercuric chlorid and formic acid against anthrax spores dried upon pieces of hide. The Bureau of Animal Industry (B. A. I.) strain of *Bacillus anthracis*, which was employed in the previously described "rod" method experiments, was used in infecting the pieces of hide.

The results of these experiments, both by cultural methods and by inoculation of animals, were entirely unsatisfactory, the reason for this being apparently that the B. A. I. strain of *Bacillus anthracis* produced spores of comparatively low virulence and low vitality.

For this reason a culture of an entirely different strain of *Bacillus anthracis* was obtained from the Army Medical School (A. M. S.) through the courtesy of Capt. Craig, and spores of this strain were used in all further experiments. Experiments were made with "naked" spores by the "rod" method and with spores dried upon pieces of hide. As the subsequent records of these experiments will show, the spores of the A. M. S. strain were found to be very much more virulent and resistant to the action of disinfectants, drying, etc., than those of the B. A. I. strain.

II. EXPERIMENTS BY ROD METHOD, USING ARMY MEDICAL SCHOOL STRAIN OF BACILLUS ANTHRACIS

The technique of these experiments was exactly the same as for those with the B. A. I. strain, except that the quantity of disinfectant per rod was made 10 c. c. instead of 5 c. c.

Experiment 5 (Table III) indicates that mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, is able to destroy anthrax spores of the A. M. S. strain in 24 hours when no organic matter is added.

TABLE III.—*Germicidal efficiency of mercuric chlorid and formic acid by the rod method, without addition of organic matter*

EXPERIMENT 5[a]

Rod No.	Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.
		Hours.	
1	Mercuric chlorid (1:4,000)+formic acid (1 per cent).....................	24	No growth.
2	Mercuric chlorid (1:5,000)+formic acid (1 per cent).....................	24	Do.
3	Control rod..	([b])	Growth.

[a] Incubated 5 days. Hydrogeu-sulphid solution used for neutralization of mercury.
[b] Not exposed.

Experiment 6, on the other hand, indicates that with an addition of defibrinated blood mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, is not able to kill spores of the A. M. S. strain in 24 hours (Table IV).

TABLE IV.—*Germicidal efficiency of mercuric chlorid, with or without formic acid, by the rod method, with and without addition of organic matter*

EXPERIMENT 6[a]

Rod No.	Disinfectant (10 c. c.) and dilution.	Quantity of blood added.	Time of exposure.	Result.
		C. c.	*Hours.*	
1	Mercuric chlorid (1:4,000)+formic acid (1 per cent)...........	None.	24	No growth.
2	Mercuric chlorid (1:4,000)+formic acid (1 per cent)...........	1	24	Growth.
3	Mercuric chlorid (1:5,000)+formic acid (1 per cent)...........	None.	24	No growth.
4	Mercuric chlorid (1:5,000)+formic acid (1 per cent)...........	1	24	Growth.
5	Mercuric chlorid (1:1,000)...................................	None.	24	No growth.
6	Mercuric chlorid (1:1,000)...................................	1	24	Growth.
7	Control rod...........................:.....................	....	([b])	Do.

[a] Incubated 3 days. Saturated aqueous solution of hydrogen sulphid used for neutralization of disinfectant.
[b] Not exposed.

In another experiment with various dilutions (Table V, experiment 7) there were 9 c. c. of disinfectant plus 1 c. c. of defibrinated blood in each tube.

Experiment 7 was repeated with the result given in Table V, experiment 8.

In experiment 9 the technique was the same as for experiment 8, the age of the culture being approximately the same (Table V, experiment 9).

TABLE V.—*Germicidal efficiency of mercuric chlorid, with and without formic acid, by the rod method,[a] with addition of defibrinated blood*

EXPERIMENT 7[b]

Rod No.	Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.
		Hours.	
1	Mercuric chlorid (1:2,000)+formic acid (1 per cent).....................	24	No growth.
2	Mercuric chlorid (1:3,000)+formic acid (1 per cent).....................	24	Growth.
3	Mercuric chlorid (1:4,000)+formic acid (1 per cent).....................	24	Do.
4	Mercuric chlorid (1:1,000)....................................	24	Do.
5	Control rod..	([c])	Do.

[a] Hydrogen-sulphid solution used to neutralize disinfectaut.
[b] The quantity of disinfectant used in experiments 7, 8, and 9 included 1 c. c. of defibrinated blood.
[c] Not exposed.

TABLE V.—*Germicidal efficiency of mercuric chlorid, with and without formic acid, by the rod method, with addition of defibrinated blood*—Continued.

EXPERIMENT 8 [a]

Rod No.	Disinfectant (10 c. c.) and dilution.	Time of exposure,	Result.
1	Mercuric chlorid (1:2,000)+formic acid (1 per cent)........................	24	Growth.
2	Mercuric chlorid (1:3,000)+formic acid (1 per cent)........................	24	Do.
3	Mercuric chlorid (1:4,000)+formic acid (1 per cent)........................	24	Do.
4	Mercuric chlorid (1:1,000)...	24	Do.
5	Control rod...	(b)	Do.

EXPERIMENT 9 [a]

1	Mercuric chlorid (1:1,000)+formic acid (1 per cent)	24	No growth.
2	Mercuric chlorid (1:2,000)+formic acid (1 per cent)........................	24	Growth.
3	Mercuric chlorid (1:3,000)+formic acid (1 per cent)	24	Do.
4	Mercuric chlorid (1:1,000)...	24	Do.
5	Control rod...	(b)	Do.

[a] The quantity of disinfectant used in experiments 7, 8, and 9 included 1 c. c. of defibrinated blood.
[b] Not exposed.

The discrepancy between the results of experiment 7 and those of experiments 8 and 9 appeared to be due to the use of a culture only 7 days old for making the spore suspension used in experiment 7, while the cultures used in experiments 8 and 9 were 17 and 22 days old, respectively.

In all these experiments spore suspensions were examined microscopically to make certain that plenty of spores were present, and it was noted that where cultures were less than 10 days old the suspensions generally contained a greater number of bacilli in relation to the spores than suspensions made from cultures 2 to 3 weeks old. The older cultures were therefore better adapted for this work.

The results of these experiments and a number of other similar experiments indicated that the A. M. S. strain of *Bacillus anthracis* was much more vigorous than the B. A. I. strain, which was used in experiments 1 to 4, and consequently was better suited for the purpose of this work. The following experiments, in which pieces of infected hide were employed, were therefore carried on with spores of the A. M. S. strain.

III. EXPERIMENTS UPON PIECES OF HIDE INFECTED WITH SPORES OF THE ARMY MEDICAL SCHOOL STRAIN OF BACILLUS ANTHRACIS WITHOUT NEUTRALIZATION OF DISINFECTANT

Some of the pieces of hide were prepared by a method essentially the same as that described by C. W. Ponder (9, 10), the details being as follows: The test preparations were made by cutting out pieces of hide so that each piece weighed about 2½ gm. Blood was drawn from the ear of a rabbit and a good-sized drop allowed to fall on the center of the hair side of each piece. Before clotting occurred a loopful of a suspension of anthrax spores was mixed thoroughly into the drop of blood.

The loop used was 3 cm. in diameter of 23-gauge platinum wire. The preparations so made were dried in the incubator 23 hours and then kept at room temperature until used.

In view of statements made by Otsuki (8) that spores of anthrax are injured by drying at 37.5° C., and that the best method of preparation is by drying them at 10° C., another lot of test preparations of hide was made as follows: Pieces of hide were cut to weigh about 2½ gm. On each piece a good-sized drop of blood from a rabbit's ear was allowed to fall and into this was mixed a loopful of a suspension of anthrax spores. This suspension was prepared by rubbing up in sterile water enough of the surface growth from a 15-day agar culture to give a suspension approximately equal in density to a 24-hour bouillon culture of *Bacillus typhosus*. These pieces of hide were placed in Petri dishes with raised covers and were dried for three days in a desiccator over sulphuric acid at a temperature of 10° C. and in a vacuum equal to about 6 cm. of mercury.

Guinea pigs were inoculated with clots from pieces of hide dried by each method. In neither case were the spores found to possess sufficient vitality to infect the animals, and it seemed evident that the methods of preparation had in some way attenuated the virulence of the spores. In view of the statement made by Roos (11) that rabbit blood is bactericidal for anthrax bacilli, while guinea pig blood is not, it seemed that the lack of virulence might be due to the use of rabbit blood. Therefore new pieces of hide were prepared, using blood from a guinea pig instead of rabbit blood as before. The pieces of hide were dried for 24 hours at 37.5° C. and then kept several days at room temperature in a dark closet. The lower drying temperature was used in later experiments. The spores in these test preparations were found to be virulent for guinea pigs, although less virulent than the original A. M. S. culture when tested shortly after it was received.

The virulence of the cultures was therefore raised by successive inoculations until a culture was obtained which killed a guinea pig in about 36 hours after subcutaneous inoculation. This culture was then employed in preparing test pieces of hide by the method above described, guinea-pig blood being used and the pieces being dried at 37.5° C. The pieces of hide so prepared were subjected to the following tests:

Each piece of hide was exposed to 25 c. c. of disinfectant for 24 hours and then soaked in 25 c. c. of saturated salt solution for 24 hours. At the end of that time the clots were scraped off and inoculated into guinea pigs. The results are given in Table VI.

TABLE VI.—*Inoculation of guinea pigs with clots from pieces of hide*

EXPERIMENT 10

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Number of clots inoculated.	Result of inoculation.
		Hours.		
23237	Mercuric chlorid (1:2,000)+formic acid (1 per cent).	24	1	Lived.
23238	Do..	24	1	Do.
23239	Mercuric chlorid (1:3,000)+formic acid (1 per cent).	24	1	Do.
23240	Do..	24	1	Do.
23241	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Do.
23242	Do..	24	1	Do.
23243	Mercuric chlorid (1:5,000)+formic acid (1 per cent).	24	1	Died in 5 days. Anthrax.
23244	Do..	24	1	Do.
23245	No disinfectant................................	(a)	1	Died in less than 48 hours. Anthrax.
23246	Do..	(a)	1	Do.

EXPERIMENT 11

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Number of clots inoculated.	Result of inoculation.
23251	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
23252	Do..	24	1	Do.
23277	Do..	24	1	Do.
23278	Do..	24	1	Do.
23279	Do..	24	1	Do.
23280	Do..	24	1	Do.
23281	Do..	24	1	Do.
23282	Do..	24	1	Do.
23250	Do..	24	2	Died in 5 days. Anthrax.
23248	No disinfectant................................	(a)	1	Died in 48 hours. Anthrax.
23249	Do..	(a)	1	Do.

EXPERIMENT 12

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Number of clots inoculated.	Result of inoculation.
24354	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
24355	Do..	24	1	Do.
23456	Do..	24	1	Do.
23457	Do..	24	1	Do.
23458	Do..	24	1	Do.
23459	Do..	24	1	Do.
23452	Do..	24	2	Do.
23453	Do..	24	2	Do.
23451	Mercuric chlorid (1:2,500)+formic acid (1 per cent).	24	5	Do.
23449	No disinfectant................................	(a)	1	Died in 48 hours. Anthrax.
23450	Do..	(a)	1	Do.

EXPERIMENT 13

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Number of clots inoculated.	Result of inoculation.
24999	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
25300	Do..	24	1	Do.
25301	Do..	24	2	Do.
25302	Do..	24	2	Do.
25303	Do..	24	4	Died in 3 days. Not anthrax.
25304	Do..	24	4	Lived.
25315	Sodium chlorid, but no disinfectant..............	(a)	1	Died in 4 days. Anthrax.
25316	Do..	(a)	1	Died in 5 days. Anthrax.

a Not exposed.

Since in experiment 10 mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, was shown to be efficient, while mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, was not, further tests were made with the lower dilution.

Ten pieces of hide were exposed for 24 hours to 25 c. c. (for each piece) of mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, and then soaked 24 hours in a saturated common-salt solution. The clots were then scraped off and inoculated into guinea pigs. In one instance two clots were inoculated into one animal; in all other cases only one clot was used (Table VI, experiment 11).

Another experiment (Table VI, experiment 12) was made in which six guinea pigs were inoculated with one clot each from pieces of hide disinfected with mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid; two guinea pigs were inoculated with two clots each from pieces similarly disinfected; and one guinea pig was inoculated with five clots from pieces of hide disinfected with mercuric chlorid, 1 to 2,500, plus 1 per cent of formic acid. As in the preceding experiments, each piece of hide was exposed for 24 hours to 25 c. c. of disinfectant and soaked in 25 c. c. of saturated common-salt solution for 24 hours, after which the clots were scraped off and inoculated under the skin of the guinea pigs.

The apparent discrepancy between experiments 11 and 12 in connection with results obtained by inoculation into guinea pigs of clots from two pieces of hide disinfected with mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, may be explained on the ground that the pieces used in the second experiment had been kept longer than those used in the first and had consequently lost virulence by continued drying. Even in experiment 11 it will be seen that the disinfectant exercised a marked influence on the virulence of the spores, since the guinea pig remained alive until five days after inoculation.

The results of these experiments are confirmed by the results of a further experiment (Table VI, experiment 13) performed later with test preparations of a different lot. This later lot was prepared in exactly the same way as the earlier ones; but the culture used for infecting the pieces of hide was obtained from a guinea pig dying a little more than 48 hours after inoculation, while the culture used for the pieces first prepared was obtained from a guinea pig dying within 36 hours after inoculation. The difference in the vitality of the spores is clearly seen in the length of time necessary to kill the guinea pigs inoculated from the check pieces. As will be seen by reference to Table VI, this time was about 48 hours for the first lot, while for the second it was from 4 to 5 days.

In order to ascertain the effect of mercuric chlorid and formic acid upon hides from the standpoint of the tanner, pieces of hide about 4 by 5 inches in size and weighing about 50 gm. each were disinfected by the Seymour-Jones method, using mercuric-chlorid dilutions of 1 to 4,000 and 1 to 2,500 plus 1 per cent of formic acid. The proportion of disinfectant used was 10 times the weight of the hide. These were examined and tanned in the Leather and Paper Laboratory of the Bureau of Chemistry. Immediately after dehairing, these pieces of hide were observed to be very much blackened, but after the full process of tanning

this was not evident, so it appeared that the coloring matter of the tanning liquid had covered up this discoloration.

Judged solely by the results of the various experiments previously described, it might seem that the Seymour-Jones method could be accepted as suitable for the disinfection of hides, provided that mercuric chlorid in a strength of 1 to 2,500 was substituted for the recommended dilution of 1 to 5,000. However, at this stage the writer's attention was called to the work of Ševčík (14), which appeared to controvert the favorable results obtained by various workers as well as his own previous results. Ševčík concluded that it is necessary to carefully neutralize the disinfectant before attempting, either by cultural methods or animal inoculation, to ascertain whether anthrax spores have been destroyed, and that the hydrogen-sulphid solution used for a short time is not sufficient to neutralize mercuric chlorid plus formic acid. The neutralizing agent which he recommended was sodium sulphid, which neutralizes both the mercury and the acid. The time which he allowed for the neutralizing process was two hours.

Ševčík's contention that the mercuric chlorid and formic acid used in the Seymour-Jones method should be neutralized by sodium sulphid in order to determine whether disinfection has been complete seemed reasonable in view of the fact that many tanners use sodium sulphid for dehairing hides; therefore, in order to verify his conclusions, the following experiments were undertaken.

IV. EXPERIMENTS UPON PIECES OF HIDE INFECTED BY SPORES OF ARMY MEDICAL SCHOOL STRAIN OF BACILLUS ANTHRACIS WITH SODIUM SULPHID AS A NEUTRALIZING AGENT

Pieces of hide were exposed to 25 c. c. of disinfectant for 24 hours, treated with 25 c. c. of saturated solution of sodium chlorid for one hour and with 25 c. c. of a 1 per cent sodium-sulphid solution for two hours. They were then washed with sterile water.

In experiment 14 (Table VII) the clots were scraped off and inoculated into guinea pigs.

TABLE VII.—*Inoculation of guinea pigs with clots from infected pieces of hide*

EXPERIMENT 14

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Result of inoculation.
		Hours.	
25522	Mercuric chlorid (1:1,000)+formic acid (1 per cent).	24	Died in 3½ days. Anthrax.
25523	Do.	24	Lived.
25524	Mercuric chlorid (1:2,500)+formic acid (1 per cent).	24	Do.
25525	Do.	24	Died in 3½ days. Anthrax.
25526	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	Died. Mixed infection.
25527	Do.	24	Died in 4 days. Anthrax.
25528	Sodium chlorid followed by sodium sulphid. No disinfectant.	(*a*)	Died in 3 days. Anthrax.
25529	Do.	(*a*)	Died. Mixed infection.

a Not exposed.

The test preparations used in experiment 14 were made as follows: Pieces of hide were cut so as to weigh about 2½ gm. A good-sized drop of guinea-pig blood was allowed to fall upon the center of each piece, and, before this clotted, a loopful of a suspension of anthrax spores was thoroughly mixed in. The suspension of spores was obtained by rubbing up in sterile water enough of the surface growth of an agar culture of *Bacillus anthracis* obtained directly from the spleen of a guinea pig (No. 25386) to give a suspension rather more dense than a 24-hour bouillon culture of *B. typhosus*. The loop employed was of No. 23 gauge platinum wire 3 mm. in diameter. The pieces of hide thus infected were dried in an electric oven at a temperature of about 45° C., in order to prevent the spores from developing into vegetative forms, which would be destroyed by the drying.

In experiment 15 (Table VIII) the test pieces of hide were prepared as follows: Pieces cut to weigh 2½ gm. were placed in a rather dense suspension of anthrax spores with hair side down. After soaking in this solution for 10 minutes they were placed in Petri dishes hair side up and allowed to dry a few minutes. Then 0.1 c. c. of the spore suspension was dropped on each piece and they were allowed to stand at room temperature for one hour. The pieces of hide were then dried in an electric oven at 43° C. for two days, the covers of the Petri dishes being tilted to one side. They were then kept at room temperature until used. After exposure to the disinfectant a considerable part of the hair with some of the underlying hide was scraped off and inoculated subcutaneously into guinea pigs, instead of inoculating blood clots as before. In other respects the technique was the same as for experiment 14.

TABLE VIII.—*Inoculation of guinea pigs with portions of hide*

EXPERIMENT 15

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Result of inoculation.
		Hours.	
25566	Mercuric chlorid (1:1,000)+formic acid (1 per cent)......	24	Died in 3½ days. Anthrax.
25567	Do...	24	Lived.
25568	Mercuric chlorid (1:2,500)+formic acid (1 per cent)	24	Died in 5 days. Anthrax.
25569	Do...	24	Do.
25570	Mercuric chlorid (1:4,000)+formic acid (1 per cent)	24	Died in 4 days. Anthrax.
25571	Do...	24	Died in 3½ days. Anthrax.
25572	Sodium chlorid followed by sodium sulphid. No disinfectant.	(a)	Do.
25573	Do...	(a)	Died in 2 days. Mixed infection.

EXPERIMENT 16

25717	Mercuric chlorid (1:500)+formic acid (1 per cent).......		Lived.
25718	Do...	24	Do.
25719	Mercuric chlorid (1:1,000)+formic acid (1 per cent).....	24	Do.
25720	Do...	24	Do.
25721	Mercuric chlorid (1:2,000)+formic acid (1 per cent).....	24	Do.
25722	Do...	24	Do.
25723	Sodium chlorid followed by sodium sulphid. No disinfectant.	(a)	Died after 4 days. Anthrax.
25724	Do...	(a)	Do.

a Not exposed.

TABLE VIII.—*Inoculation of guinea pigs with portions of hide*—Continued

EXPERIMENT 17

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Result of inoculation.
		Hours.	
24598	Mercuric chlorid (1:500)+formic acid (1 per cent).......	24	Lived.
24599	Do...	24	Died after 7 days. Anthrax.
25725	Mercuric chlorid (1:1,000)+formic acid (1 per cent).....	24	Lived.
25726	Do...	24	Do.
25727	Mercuric chlorid (1:2,000)+formic acid (1 per cent).....	24	Died after 6 days. Anthrax.
25728	Do...	24	Lived.
25729	Sodium chlorid followed by sodium sulphid. No disinfectant.	(a)	Died after 3 days. Mixed infection.
25730	Do...	(a)	Died after 5 days. Anthrax.

EXPERIMENT 18

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Result of inoculation.
27221	Mercuric chlorid (1:250)+formic acid (1 per cent).......	24	Died after 4 days. Anthrax.
27222	Do...	24	Died after 6 days. Not anthrax.
27223	Mercuric chlorid (1:500)+formic acid (1 per cent).......	24	Died after 5 days. Anthrax.
27224	Do...	24	Died after 6 days. Anthrax.
27225	Mercuric chlorid (1:1,000)+formic acid (1 per cent).....	24	Died after 5 days. Anthrax.
27226	Do...	24	Lived.
27227	Mercuric chlorid (1:2,000)+formic acid (1 per cent).....	24	Died after 5 days. Anthrax.
27228	Do...	24	Died after 6 days. Anthrax.
27231	Sodium chlorid followed by sodium sulphid. No disinfectant.	(a)	Died after 5 days. Anthrax.
27232	Do...	(a)	Died. Mixed infection.

a Not exposed.

Experiment 16 (Table VIII) was similar to the preceding, except that the pieces of hide used were dried for three instead of two days. A culture from the heart blood of guinea pig 25515 was used in making the spore suspension.

Apparently the added duration of drying had an injurious action upon the spores. It should be noted, however, that cultures from one guinea pig (No. 25386) were used in preparing material in experiments 14 and 15, while the test preparations used in experiment 16 were infected by a culture derived from a different animal.

The available cultures from the same source as those used in preparing material for experiments 14 and 15 were now 1 month old. In experiment 17 (Table VIII) one of these was used in infecting pieces of hide in the following way: Pieces of hide of 2½ gm. weight were soaked in a suspension of anthrax spores for 10 minutes; then one-tenth c. c. of suspension was dropped on each, and the pieces of hide were dried in an electric oven at 43° C. for 24 hours and then kept at room temperature for 24 hours before use. The covers of the Petri dishes containing the pieces of hide were kept raised during all of this time.

The results of this experiment seem to indicate that cultures derived from one animal (guinea pig 25386) yielded spores of very great resisting power as compared with cultures from another animal (guinea pig 25515). The irregularities which will be noted in experiment 17 are probably due to the age of the culture used.

A further series of experiments having given unsatisfactory results, it was deemed advisable to undertake comparative tests of infected pieces of hide prepared by several different methods.

Further experiments were thereupon made to compare the infectivity of pieces of hide dried (1) in an electric oven at 44° C. for 40 hours; (2) in an incubator at 37° C. for 24 hours (spores in blood clots); and (3) in a desiccator over sulphuric acid at a temperature of about 10° C., the desiccator being exhausted of air down to a pressure of about 6 cm. of mercury, time of drying, 48 hours.

Of the above only those pieces dried at a low temperature proved infectious, the guinea pig inoculated dying after one week. As a guinea pig inoculated by pure culture also remained alive for a week, it seemed that the process of drying at 10° C. in a vacuum over sulphuric acid had not appreciably diminished the virulence of the spores. This process was therefore used in the preparation of all further test pieces of hide.

Previous experiments had shown a difference between the two strains of guinea pigs which had been used in these experiments, one strain being much more susceptible to infection by anthrax than the other. The comparatively low virulence of the pure culture mentioned above seemed to be due to passage through the less resistant strain of guinea pigs. Beginning, therefore, with a culture which had not been so treated, successive inoculations were made with the more resistant strain of guinea pigs until cultures of satisfactory virulence and vitality were obtained.

V. EXPERIMENTS UPON INFECTED PIECES OF HIDE DRIED AT 10° C.

A lot of pieces of hide were prepared as follows: Pieces of $2\frac{1}{2}$ gm. in weight were washed and dried. These were infected by a suspension made from a 7-day agar culture, in the following manner: Pieces were placed in the suspension, hair side down, and allowed to soak for 10 minutes, and then 0.2 c. c. of the suspension was dropped on each. These pieces were left in Petri dishes in the ice box for half an hour with covers of dishes on. At the end of that time the dishes were placed in a desiccator over sulphuric acid and the covers raised. The desiccator was then exhausted of air and put into the ice box, where it remained 48 hours at a temperature of 10° C. The pieces of hide were then removed and kept at room temperature until used. A guinea pig inoculated with the pure culture used for infecting these pieces of hide died in four days. Using the pieces of hide prepared as described, the following experiments were performed:

In experiment 18, pieces of hide were exposed to the disinfectant for 24 hours, followed by a saturated salt solution for 1 hour. They were then treated with a 1 per cent sodium-sulphid solution for 2 hours and washed with sterile water. Material was then scraped from the surface of each and inoculated into a guinea pig. The results are given in Table VIII, experiment 18.

In this experiment, as in those of similar character preceding it, neutralization of the disinfectant by sodium sulphid was done within a comparatively few hours after the process of disinfection was complete. In view of the strong dilution (1 to 250) found to be inefficient under these circumstances, no further attempt was made to find a dilution strong enough to disinfect, with neutralization afterward. Instead of this, an attempt was now made to determine how long spores remained viable after treatment of the pieces of hide by much weaker dilutions of mercuric chlorid plus formic acid. This seemed worth while because the Seymour-Jones method was originally proposed to be employed at foreign ports, and in a voyage of ordinary length a considerable time would thus elapse between the time of disinfection and time of arrival at destination.

In experiment 19 (Table IX) a number of pieces of hide were exposed for 24 hours to mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, treated with saturated common salt for 1 hour, and then laid aside and at intervals treated with sodium sulphid and inoculated into guinea pigs. In each case they were treated with 1 per cent of sodium sulphid for 2 hours and washed with sterile distilled water. Material was then scraped from each piece and inoculated subcutaneously into a guinea pig.

TABLE IX.—*Inoculation of guinea pigs with infected portions of hide* ·

EXPERIMENT 19 [a]

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Time before treatment with sodium sulphid.	Result of inoculation.
		Hours.	Days.	
27229	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
27229	Do....................................	24	1	Do.
27257	Do....................................	24	2	Died after 6 days. Anthrax.
27258	Do....................................	24	2	Died. Pneumonia.
27259	Do....................................	24	3	Died after 6½ days. Anthrax.
27260	Do....................................	24	3	Lived.
27261	Do....................................	24	4	Died after 10 days. Anthrax.
27262	Do....................................	24	4	Died. Pneumonia.

EXPERIMENT 20 [b]

		Hours.	Days.	
27521	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
27522	Do....................................	24	1	Do.
27525	Do....................................	24	3	Died after 6 days. Anthrax.
27526	Do....................................	24	3	Lived.
27531	Do....................................	24	6	Died after 4 days. Anthrax.
27532	Do....................................	24	6	Died after 5 days. Anthrax.
			Weeks.	
28004	Do....................................	24	2	Lived.
28005	Do....................................	24	2	Do.

[a] Control guinea pig died of anthrax in 5 days.
[b] Control guinea pig died of anthrax in 7 days.

TABLE IX.—*Inoculation of guinea pigs with infected portions of hide*—Continued

EXPERIMENT 21 [a]

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Time before treatment with sodium sulphid.	Result of inoculation.
		Hours.	Days.	
28006	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
28007	Do..	24	1	Do.
28010	Do..	24	4	Died after 9 days.　Anthrax.
28011	Do..	24	4	Died after 3 days.　Anthrax.
28056	Do..	24	9	Died after 7 days.　Anthrax.
28057	Do..	24	9	Died after 6 days.　Anthrax.
			Weeks.	
28072	Do..	24	2	Do.
28073	Do..	24	2	Died after 8 days.　Anthrax.

EXPERIMENT 22 [b]

		Hours.	Days.	
28012	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Died after 9 days.　Anthrax.
28013	Do..	24	1	Lived.
28008	Mercuric chlorid (1:2,500)+formic acid (1 per cent).	24	1	Died after 13 days. Anthrax.
28009	Do..	24	1	Lived.
28052	Do..	24	2	Do.
28053	Do..	24	2	Do.
28054	Do..	24	4	Died after 6 days.　Anthrax.
28055	Do..	24	4	Lived.
28058	Do..	24	6	Do.
28059	Do..	24	6	Do.

[a] Control guinea pig died of anthrax in 4 days.
[b] Control guinea pig died of mixed infection.

The irregular results noted above might be due to variation in the extent of infection of the various pieces of hide.

In another experiment with similar technique, except that the pieces of hide were infected at a different time and by a different culture, the results were as given in Table IX, experiment 20.

In experiment 21 (Table IX), also, the procedure was the same as in experiment 19, except that the test pieces of hide were infected by a different culture.

Experiment 22 (Table IX) was similar to the preceding experiments, except in the use of a stronger dilution of the disinfectant.

In connection with experiments 20, 21, and 22 part of the material scraped from the pieces of hide was plated out to determine whether sterilization had been accomplished. Growth of some kind was obtained in every instance, although *Bacillus anthracis* was isolated in only about one-third of the cases. In one instance *B. anthracis* was recovered from material which failed to cause anthrax when inoculated into guinea pigs, but on the other hand, one guinea pig died from anthrax after inoculation with material which failed to yield *B. anthracis* by the plate method.

HYDROCHLORIC ACID AND SODIUM CHLORID

In view of the apparent inefficiency of the Seymour-Jones method and the favorable results reported by various workers using the Schattenfroh method, experiments were now undertaken to determine the germicidal power of hydrochloric acid and sodium chlorid against anthrax spores, both as "naked" spores and as contained on and in infected pieces of hide. The Schattenfroh method (12) as described by Prof. Schattenfroh consists of immersion of hides in solutions of hydrochloric acid and common salt, the proportions recommended varying according to temperature. The proportions recommended for use at room temperature are 2 per cent of hydrochloric acid plus 10 per cent of sodium chlorid, with the time of exposure 48 hours. At higher temperatures less of the acid is needed and the time of exposure is shortened, but inasmuch as special apparatus would be needed to maintain these higher temperatures it seemed that disinfection at these higher temperatures could be disregarded as being of little practical significance.

The experiments here described were therefore carried on at room temperature. In all cases dilutions were calculated upon the percentage of absolute hydrochloric acid, not upon the percentage of "concentrated hydrochloric acid." In accordance with Schattenfroh's recommendations, a sodium-carbonate solution was used after exposure to the disinfectant, in order to neutralize the hydrochloric acid.

I. EXPERIMENTS BY THE ROD METHOD, USING SPORES OF BACILLUS ANTHRACIS

A series of experiments was first made by the rod method, using various proportions of hydrochloric acid and sodium chlorid. The time of exposure in each case was 24 hours, and rods were washed with a 2 per cent solution of sodium carbonate to neutralize the hydrochloric acid. Experiment 23 (Table X) was made without the addition of organic matter; experiment 24 (Table X) was made with the addition of 1 c. c. of defibrinated blood to 9 c. c. of disinfectant in each tube. The results are given in Table X, together with the results of an experiment upon mercuric chlorid, alone and with acetic acid and formic acid, which was made at the same time, and with rods infected by the same spore suspension. This suspension was rather heavier than usual. In experiment 24 the hydrochloric-acid rods were washed in a 20 c. c. sodium-carbonate solution for one minute, and the mercuric chlorid rods in a 20 c. c. saturated hydrogen sulphid for one minute.

Table X.—*Germicidal efficiency of hydrochloric acid plus sodium chlorid and mercuric chlorid, with and without formic acid, and with acetic acid, by the rod method, without addition of organic matter[a]*

EXPERIMENT 23

Rod No.	Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.
		Hours.	
1	Hydrochloric acid (1 per cent)+sodium chlorid (5 per cent).............	24	No growth.
2	Hydrochloric acid (2 per cent)+sodium chlorid (5 per cent).............	24	Do.
3	Hydrochloric acid (3 per cent)+sodium chlorid (5 per cent).............	24	Do.
4	Hydrochloric acid (4 per cent)+sodium chlorid (5 per cent).............	24	Do.
5	Hydrochloric acid (5 per cent)+sodium chlorid (5 per cent).............	24	Do.
6	Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent).............	24	Do.
7	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).............	24	Do.
8	Hydrochloric acid (3 per cent)+sodium chlorid (10 per cent).............	24	Do.
9	Hydrochloric acid (4 per cent)+sodium chlorid (10 per cent).............	24	Do.
10	Hydrochloric acid (5 per cent)+sodium chlorid (10 per cent).............	24	Do.
11	Hydrochloric acid (1 per cent)+sodium chlorid (15 per cent).............	24	Do.
12	Hydrochloric acid (2 per cent)+sodium chlorid (15 per cent).............	24	Do.
13	Hydrochloric acid (3 per cent)+sodium chlorid (15 per cent).............	24	Do.
14	Hydrochloric acid (4 per cent)+sodium chlorid (15 per cent).............	24	Do.
15	Hydrochloric acid (5 per cent)+sodium chlorid (15 per cent).............	24	Do.
16	Hydrochloric acid (1 per cent)+sodium chlorid (20 per cent).............	24	Do.
17	Hydrochloric acid (2 per cent)+sodium chlorid (20 per cent).............	24	Do.
18	Hydrochloric acid (3 per cent)+sodium chlorid (20 per cent).............	24	Do.
19	Hydrochloric acid (4 per cent)+sodium chlorid (20 per cent).............	24	Do.
20	Hydrochloric acid (5 per cent)+sodium chlorid (20 per cent).............	24	No.
21	Control rod...	(b)	Growth.

EXPERIMENT 24[c]

1	Hydrochloric acid (1 per cent)+sodium chlorid (5 per cent).............	24	Growth.
2	Hydrochloric acid (2 per cent)+sodium chlorid (5 per cent).............	24	Do.
3	Hydrochloric acid (3 per cent)+sodium chlorid (5 per cent).............	24	Do.
4	Hydrochloric acid (4 per cent)+sodium chlorid (5 per cent).............	24	Do.
5	Hydrochloric acid (5 per cent)+sodium chlorid (5 per cent).............	24	Do.
6	Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent).............	24	Do.
7	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).............	24	Do.
8	Hydrochloric acid (3 per cent)+sodium chlorid (10 per cent).............	24	No growth.
9	Hydrochloric acid (4 per cent)+sodium chlorid (10 per cent).............	24	Do.
10	Hydrochloric acid (5 per cent)+sodium chlorid (10 per cent).............	24	Do.
11	Hydrochloric acid (1 per cent)+sodium chlorid (15 per cent).............	24	Growth.
12	Hydrochloric acid (2 per cent)+sodium chlorid (15 per cent).............	24	Do.
13	Hydrochloric acid (3 per cent)+sodium chlorid (15 per cent).............	24	No growth.
14	Hydrochloric acid (4 per cent)+sodium chlorid (15 per cent).............	24	Do.
15	Hydrochloric acid (5 per cent)+sodium chlorid (15 per cent).............	24	Do.
16	Hydrochloric acid (1 per cent)+sodium chlorid (20 per cent).............	24	Do.
17	Hydrochloric acid (2 per cent)+sodium chlorid (20 per cent).............	24	Growth.
18	Hydrochloric acid (3 per cent)+sodium chlorid (20 per cent).............	24	No growth.
19	Hydrochloric acid (4 per cent)+sodium chlorid (20 per cent).............	24	Growth.
20	Hydrochloric acid (5 per cent)+sodium chlorid (20 per cent).............	24	No growth.
21	Mercuric chlorid (1:500) alone......................................	24	Growth.
22	Mercuric chlorid (1:1,000)+acetic acid (1 per cent)	24	Do.
23	Mercuric chlorid (1:2,000)+acetic acid (1 per cent)	24	Do.
24	Mercuric chlorid (1:3,000)+acetic acid (1 per cent)	24	Do.
25	Mercuric chlorid (1:1,000)+formic acid (1 per cent)	24	Do.
26	Mercuric chlorid (1:2,000)+formic acid (1 per cent)	24	Do.
27	Mercuric chlorid (1:3,000)+formic acid (1 per cent)	24	Do.
28	Control rod...	(b)	Do.

EXPERIMENT 25 [c]

1	Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent).............	24	No growth.
2	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).............	24	Do.
3	Hydrochloric acid (3 per cent)+sodium chlorid (10 per cent).............	24	Do.
4	Hydrochloric acid (4 per cent)+sodium chlorid (10 per cent).............	24	Do.
5	Hydrochloric acid (5 per cent)+sodium chlorid (10 per cent).............	24	Do.
6	Hydrochloric acid (1 per cent)+sodium chlorid (15 per cent).............	24	Do.
7	Hydrochloric acid (2 per cent)+sodium chlorid (15 per cent).............	24	Do.
8	Hydrochloric acid (3 per cent)+sodium chlorid (15 per cent).............	24	Do.
9	Hydrochloric acid (4 per cent)+sodium chlorid (15 per cent).............	24	Do.
10	Hydrochloric acid (5 per cent)+sodium chlorid (15 per cent).............	24	Do.
11	Hydrochloric acid (1 per cent)+sodium chlorid (20 per cent).............	24	Do.
12	Hydrochloric acid (2 per cent)+sodium chlorid (20 per cent).............	24	Do.
13	Hydrochloric acid (3 per cent)+sodium chlorid (20 per cent).............	24	Do.
14	Hydrochloric acid (4 per cent)+sodium chlorid (20 per cent).............	24	Do.

[a] Percentage of hydrochloric acid means percentage of absolute hydrochloric acid.
[b] Not exposed.
[c] The quantity of disinfectant used (10 c. c.) included 1 c. c. of defibrinated blood.

TABLE X.—*Germicidal efficiency of hydrochloric acid plus sodium chlorid and mercuric chlorid, with and without formic acid, and with acetic acid, by the rod method, without addition of organic matter*—Continued

EXPERIMENT 25—continued

Rod No.	Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.
		Hours.	
15	Hydrochloric acid (5 per cent)+sodium chlorid (20 per cent).............	24	No growth.
16	Mercuric chlorid (1:500) alone...................................	24	Growth.
17	Mercuric chlorid (1:1,000) alone.................................	24	Do.
18	Mercuric chlorid (1:1,000)+acetic acid (1 per cent)................	24	Do.
19	Mercuric chlorid (1:2,000)+acetic acid (1 per cent)................	24	Do.
20	Mercuric chlorid (1:3,000)+acetic acid (1 per cent)................	24	Do.
21	Mercuric chlorid (1:1,000)+formic acid (1 per cent)...............	24	No growth.
22	Mercuric chlorid (1:2,000)+formic acid (1 per cent)...............	24	Do.
23	Mercuric chlorid (1:3,000)+formic acid (1 per cent)...............	24	Growth.
24	Control rod..	(a)	Do.

a Not exposed.

A similar experiment (Table X, experiment 25) was made with rods infected by a spore suspension of about the same density as a 24-hour bouillon culture of *Bacillus typhosus*.

In experiments 26 and 27 (Table XI) are shown a comparison of hydrochloric acid and common salt with several other disinfectants, all with 24-hour exposure. Three rods were used with each dilution, showing the result, respectively, when no defibrinated blood was added, with ½ c. c. of blood added to each tube and with 1 c. c. of blood added to each tube. The hydrochloric-acid rods were washed with a 2 per cent sodium-carbonate solution, the mercuric-chlorid rods with a saturated hydrogen-sulphid solution, and the formalin and carbolic-acid rods with distilled water.

TABLE XI.—*Germicidal efficiency of hydrochloric acid plus sodium chlorid, formalin, phenol, and mercuric chlorid, with and without formic acid, by the rod method*

EXPERIMENT 26

Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.		
		No blood added.	½ c. c. blood added.	1 c. c. blood added.
	Hours.			
Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent).	24	No growth..	No growth..	No growth.
Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).	24	.....do......	.....do......	Do.
Hydrochloric acid (3 per cent)+sodium chlorid (10 per cent).	24	.....do......	.....do......	Do.
Mercuric chlorid (1:1,000)+formic acid (1 per cent).	24	.....do......	.....do......	Growth.
Mercuric chlorid (1:2,000)+formic acid (1 per cent).	24	.....do......	.....do......	Do.
Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	.....do......	Growth.....	Do.
Mercuric chlorid (1:8,000)+formic acid (1 per cent).	24	.....do..:...	.....do......	Do.
Mercuric chlorid (1:16,000)+formic acid (1 per cent).	24	.....do......	.....do......	Do.
Formalin (1:50).................................	24	.....do......	No growth..	Do.
Formalin (1:100)................................	24	.....do......	Growth.....	Do.
Formalin (1:250)................................	24	Growth.....	.....do......	Do.
Formalin (1:1,000)..............................	24	.....do......	.....do......	Do.
Phenol (5 per cent).............................	24	.....do......	.....do......	Do.

TABLE XI.—*Germicidal efficiency of hydrochloric acid plus sodium chlorid, formalin, phenol, and mercuric chlorid, with and without formic acid, by rod method*—Continued

EXPERIMENT 27

Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.	
		½ c. c. blood added.	1 c. c. blood added.
	Hours.		
Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent)....	24	No growth..	No growth.
Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent)....	24	.....do......	Do.
Mercuric chlorid (1:1,000)+formic acid (1 per cent).............	24	.....do......	Do.
Mercuric chlorid (1:2,000)+formic acid (1 per cent).............	24	.....do......	Growth.
Mercuric chlorid (1:4,000)+formic acid (1 per cent).............	24	Growth.....	Do.
Mercuric chlorid (1:6,000)+formic acid (1 per cent).............	24	.....do......	Do.
Mercuric chlorid (1:8,000)+formic acid (1 per cent).............	24	.....do......	Do.
Formalin (1:50)..	24	No growth..	Do.
Formalin (1:100)...	24	Growth.....	Do.
Formalin (1:200)...	24	.....do......	Do.

The technique of experiment 27 was the same as that of No. 26. In this case two rods were used with each dilution, showing results with ½ c. c. and 1 c. c. of defibrinated blood.

II. EXPERIMENTS UPON PIECES OF HIDE INFECTED WITH SPORES OF BACILLUS ANTHRACIS

In experiment 28 (Table XII) a 2 per cent hydrochloric-acid solution plus 10 per cent of sodium chlorid was used with a 48-hour exposure, 25 c. c. of the disinfectant being used for each piece of hide. After exposure the pieces of hide were soaked for 15 minutes in a 3 per cent solution of sodium carbonate (25 c. c. for each). The pieces of hide used were prepared by the method given by Ponder (9, 10) and were part of the same lot as the pieces used in experiment 14. After disinfection the clots were scraped off and inoculated subcutaneously into guinea pigs.

TABLE XII.—*Inoculation of guinea pigs with clots scraped from pieces of hide*

EXPERIMENT 28

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Number of clots used.	Result of inoculation.
		Hours.		
25556	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).	48	2	Lived.
25557	Do...	48	2	Do.
25558	Do...	48	1	Do.
25559	Do...	48	1	Do.
25560	Do...	48	1	Do.
25561	Do...	48	1	Do.
25562	Do...	48	1	Do.
25563	Do...	48	1	Do.
25554	No disinfectant.....................................	(*a*)	....	Died. Anthrax.
25555	Do...	(*a*)	....	Do.

a Not exposed.

Experiment 29 (Table XIII) was made as follows: Pieces of hide were prepared by soaking in spore suspension and then drying in an electric oven. Details given in connection with experiment 15 will apply to this experiment. The technique otherwise was the same as that of experiment 28. Material was scraped from the surface of each piece and inoculated subcutaneously into guinea pigs.

TABLE XIII.—*Inoculation of guinea pigs with material scraped from pieces of hide*

EXPERIMENT 29

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Result of inoculation.
		Hours.	
25710	Hydrochloric acid (2 per cent) + sodium chlorid (10 per cent).	48	Lived.
25711	Do	48	Do.
25712	Do	48	Do.
25713	Do	48	Do.
25714	Do	48	Do.
25715	No disinfectant	(a)	Died in 3½ days. Anthrax.
25716	Do	(a)	Died in 6 days. Anthrax.

EXPERIMENT 30

25733	Hydrochloric acid (2 per cent) + sodium chlorid (10 per cent).	48	Lived.
25734	Do	48	Do.
25735	Do	48	Do.
25736	Do	48	Do.
25737	Do	48	Do.
25738	Do	48	Do.
25739	Do	48	Do.
25740	Do	48	Do.
25741	Do	48	Do.
24742	Do	48	Do.
25729	No disinfectant	(a)	Died. Mixed infection.
25730	Do	(a)	Died after 5 days. Anthrax.

EXPERIMENT 31

27593	Hydrochloric acid (2 per cent) + sodium chlorid (10 per cent).	48	Lived.
27594	Do	48	Do.
27595	Do	48	Do.
27596	Do	48	Do.
27597	Do	48	Do.
27598	Do	48	Do.
27599	Do	48	Do.
28000	Do	48	Do.
28001	No disinfectant	(a)	Died after 4 days. Anthrax.
28002	Do	(a)	Died after 3 days. Anthrax.

a Not exposed.

Experiment 30 (Table XIII) was made upon pieces of hide prepared in the same way but infected with a different culture.

In experiment 31 (Table XIII) the pieces of hide were prepared by soaking in spore suspension and drying them over sulphuric acid in a vacuum at 10° C. for 48 hours. As before, each piece of hide after disinfection was immersed for 15 minutes in 25 c. c. of a 3 per cent sodium-carbonate solution.

In connection with experiment 31 an attempt was made to determine the efficiency of disinfection by plating out material from the piece of

hide. The plates showed no growth even after three days' incubation; hence, it seemed that the hydrochloric acid and sodium chlorid had destroyed the anthrax spores and all other organisms as well.

Experiments 32 and 33 (Table XIV) show comparative tests of the Seymour-Jones and Schattenfroh methods upon pieces of hide of the same lot. These were prepared by the method described under experiment 31. The greatest possible care was taken to neutralize the disinfectant, so far as the Schattenfroh method was concerned. Sodium sulphid was used both for Seymour-Jones and Schattenfroh pieces, because it seemed possible that the depilatory action of the sodium sulphid might bring up undisinfected spores from the depths of the hair follicles. A number of pieces of disinfected hide were kept several days and then treated with the neutralizing agent.

TABLE XIV.—*Comparison of Seymour-Jones and Schattenfroh methods of disinfecting hides*

EXPERIMENT 32

Guinea pig No.	Disinfectant(25 c. c.)and dilution.	Neutralizing solution and time required.	Time of exposure.	Result of inoculation.
			Hours.	
28556	Hydrochloric acid (2 per cent)+ sodium chlorid (10 per cent).	Sodium carbonate (2 per cent), ½ hour.	48	Lived.
28557	Do..............................	....do............................	48	Do.
28558	Do..............................	Potassium hydroxid (0.5 per cent), 2 hours.	48	Do.
28559	Do..............................	....do............................	48	Do.
28560	Do..............................	Sodium sulphid (1 per cent), 2 hours.	48	Do.
28561	Do..............................	....do............................	48	Do.
28562	Mercuric chlorid (1:2,500)+formic acid (1 per cent).	....do............................	24	Do.
28563	Do..............................	....do............................	24	Do.
		Neutralization 4 days later.		
28588	Mercuric chlorid (1:2,500)+formic acid (1 per cent).	Sodium sulphid (1 per cent), 2 hours.	24	Died. Anthrax.
28589	Do..............................	....do............................	24	Do.
28590	Hydrochloric acid (2 per cent)+ sodium chlorid (10 per cent).	....do............................	48	Lived.
28591	Do..............................	....do............................	48	Do.

EXPERIMENT 33

28735	Hydrochloric acid (2 per cent)+ sodium chlorid (10 per cent).	Sodium carbonate (2 per cent), ½ hour.	48	Lived.
28736	Do..............................	....do............................	48	Do.
28737	Do..............................	Potassium hydroxid (0.5 per cent), 2 hours.	48	Do.
28738	Do..............................	....do............................	48	Do.
28739	Do..............................	Sodium sulphid (1 per cent), 2 hours.	48	Do.
28740	Do..............................	....do............................	48	Do.
28729	Mercuric chlorid (1: 2,500)+formic acid (1 per cent).	....do............................	24	Do.
28730	Do..............................	....do............................	24	Died. Anthrax.
		Neutralization 4 days later.		
28773	Mercuric chlorid (1: 2,500)+formic acid (1 per cent).	Sodium sulphid (1 per cent), 2 hours.	24	Died. Anthrax.
28774	Do..............................	....do............................	24	Lived.
28771	Hydrochloric acid (2 per cent)+ sodium chlorid (10 per cent).	....do............................	48	Do.
28772	Do..............................	....do............................	48	Do.

As a part of experiment 32, plates were made from the material scraped off the pieces of hide. In every instance the plates made from material treated by 2 per cent of hydrochloric acid and 10 per cent of sodium chlorid were sterile. On the other hand, growth was observed on all the plates from material exposed to mercuric chlorid and formic acid.

In this experiment, as in several of the last few experiments described in the previous discussion of the Seymour-Jones method, it will be noted that material from pieces of hide exposed to mercuric chlorid and formic acid and treated shortly after completion of the disinfection with sodium sulphid failed to kill guinea pigs into which it was inoculated. On the other hand, material from pieces of hide allowed to stand for several days before using sodium sulphid caused guinea pigs to die from anthrax. It was noted that the depilatory action of the sodium sulphid was far more complete in the case of the pieces of hide which had been kept for several days after disinfection before treatment with the sulphid. The results of plating, as before mentioned, showed that disinfection was not complete; therefore it seems probable that the more extensive depilatory action of the sodium sulphid upon pieces which had stood for some time brought up from the depths of the hair follicles spores which had been practically untouched by the disinfectant. It also seems possible that there had been some development and multiplication of these uninjured organisms during the period of waiting.

It should be noted that in the preparation of the pieces of hide used in all the above-mentioned experiments particular care was taken to secure penetration of the spores into the pieces of hide. In order to accomplish this, the pieces of hide after being infected by spore suspensions were placed in closed Petri dishes and kept in the ice box for four or five hours before the drying process was begun.

As will be seen by reference to Table XIII, the Schattenfroh method was entirely successful in every instance, and the results of plating showed that actual sterilization was accomplished.

Experiment 33 (Table XIV) was exactly similar to the preceding experiment except that the pieces of hide used were infected by spores derived from a different culture. The method of preparation was the same as that described under experiment 31.

In this experiment, as in the preceding one, the efficiency of the disinfectants was tested by plating out material from the pieces of hide. The results obtained varied from the results of experiment 32 in that a few colonies were found on two plates from material treated with hydrochloric acid and salt, while all other plates from similar material were sterile. One plate from material neutralized by 0.5 per cent of potassium hydrate showed two colonies, while the other, from material neutralized by sodium carbonate, showed one colony. In none of the three was *Bacillus anthracis* the organism present. Therefore, although hydro-

chloric acid and salt did not accomplish actual sterilization in every instance, it did destroy anthrax spores in every instance.

Several pieces of hide about 50 gm. weight each were exposed to 2 per cent of hydrochloric acid plus 10 per cent of sodium chlorid for 48 hours and thoroughly washed with 3 per cent sodium-carbonate solution. They were then examined and tanned in the Leather and Paper Laboratory of the Bureau of Chemistry, along with pieces of hide which had been treated by other disinfectants. This work was in charge of Mr. F. P. Veitch, and the result is shown in his memorandum on page 91.

OTHER DISINFECTANTS

Bacteriological tests were made with formalin and phenol, and pieces of hide treated by these disinfectants were examined and tanned in the Leather and Paper Laboratory of the Bureau of Chemistry. Without going into details it may be stated that, so far as could be determined by the limited number of tests, 2½ per cent of formalin is efficient bacteriologically both against anthrax spores and against other organisms, while 5 per cent of phenol is fairly efficient against non-spore-bearing organisms, but is practically useless against anthrax spores. It should be noted also that pieces of hide disinfected by formalin in 2½ per cent solution were so seriously affected by the disinfectant that it was almost impossible to tan them, while pieces treated with carbolic acid were uninjured.

A few tests were made of the germicidal efficiency of mercuric-chlorid solutions saturated with sodium chlorid. It was found that this combination is, if anything, not as efficient as mercuric chlorid alone. This is presumably due to interference of the salt with the ionization of the mercuric chlorid, as the work of Krönig and Paul (6) quite clearly indicates.

During the course of the investigations herein recorded, the writer noted considerable variations in the vitality and virulence of anthrax spores from different sources. It was also noted that the processes employed in infecting and drying test preparations exercised a variable influence upon the vitality of the spores. In view of these variations, it was found to be necessary to repeat the tests many times, and in order to test the various methods as thoroughly as possible, every effort was made to maintain at the highest possible point the vitality and virulence of the spores used in test preparations and to make sure of the presence of a considerable number of such spores upon each test preparation.

It seems likely that anthrax spores occurring upon naturally infected hides might in many cases be present in much smaller numbers and possess far less vitality and virulence than those used in the experiments. However, in view of the results obtained by Ševčík (14) and

others working with naturally infected hides, it is evident that the spores upon such hides frequently possess very high vitality and virulence. Therefore it seems that the only safe rule to follow is to use only such disinfectants and such methods of disinfection as have been found efficient against spores of maximum vitality and virulence.

SUMMARY AND CONCLUSIONS

(1) THE SEYMOUR-JONES METHOD.—The strength of disinfectant originally recommended by Seymour-Jones (mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid) was not found to be efficient, even without neutralization of the disinfectant. A stronger dilution, 1 to 2,500, plus 1 per cent of formic acid, was found to be efficient where no neutralization was attempted. The latter strength was not sufficient, however, to prevent fatal infection of guinea pigs by disinfected material when the disinfectant was neutralized by a 1 per cent sodium-sulphid solution three or four days after the completion of the process of disinfection. No infection was caused by the inoculation of material which had been kept a week or more after disinfection. It seems, therefore, that the Seymour-Jones method might be employed with dilutions of mercuric chlorid, 1 to 2,500, plus 1 per cent of formic acid, provided the treated hides are not to be subjected within a week or two to the action of any substance which will neutralize the disinfectant. This would be the case, for instance, if hides were disinfected at foreign ports before shipment to this country.

(2) THE SCHATTENFROH METHOD.—Hydrochloric acid and sodium chlorid in the proportions of 2 per cent of the acid and 10 per cent of the salt and with 48 hours' exposure have proved efficient in every instance. Consequently from the bacteriological standpoint the Schattenfroh method seems to be entirely satisfactory. This conclusion is supported not only by this work but by the exhaustive researches of Gegenbauer and Reichel (3) and Hilgermann and Marmann (4). The recently published work of Sevčík (15) is not so favorable to the Schattenfroh method as that of the investigators previously mentioned. He finds that complete disinfection can be accomplished when the hides worked with are thin. But when the hides are thick and heavily infected, he was able, after very thorough neutralization, to extract from pieces of the treated hides anthrax spores which were virulent for mice, and in some instances for guinea pigs, even after exposure to a solution of 2 per cent of hydrochloric acid plus 10 per cent of sodium chlorid for 7 days.

Although in view of the above-mentioned results the Schattenfroh method can not be regarded as perfect, it nevertheless seems to be far superior to other methods and well worth a trial as a standard method for the disinfection of hides.

(3) EFFECT OF DISINFECTION UPON HIDES AS REGARDS TANNING.— Mr. F. P. Veitch, Chemist in Charge of the Leather and Paper Laboratory

of the Bureau of Chemistry, has been kind enough to furnish the following memorandum in regard to the tanning of small pieces of normal hide treated by the Seymour-Jones and Schattenfroh processes of disinfection.

No marked differences in color were noted among the various pieces of tanned leather. Slight differences, due to difference in thickness, were noted in pliability, but these did not appear to be connected with the disinfecting treatment. No marked difference could be detected in the appearance of the grain of the leather. All the pieces cracked when severely bent, owing probably to excessive tannin in the grain of the leathers. The treated leathers did not display more pronounced cracking than those which were not treated. Microscopical examination of the hide fibers after deliming and of the leather fibers after tanning shows no marked differences among the several pieces of hide.

The results in general seem to indicate that the several treatments have not injured the hides. The evidence, however, is not sufficient to permit of definite conclusions being drawn at this time. More extended work in commercial tannery, using whole hides, has been planned to determine definitely whether any of the disinfectants result in the production of inferior leather. Since tanning is a slow process, it will require from nine months to a year to secure these data.

Mr. Veitch also states that all the leathers gave reactions for chlorids, but that the leathers treated with disinfectants apparently contained larger amounts of chlorids than the other leathers.

It seems, then, so far as the evidence at hand permits any conclusion at all, that neither the Seymour-Jones method nor the Schattenfroh method exerts any injurious effect upon hides or leather.

LITERATURE CITED

(1) Anthrax Investigation Board for Bradford [England] and District.
   [1908.] Third Annual Report. [1907]/08, 15 p.
(2) Eurich, F. W.
   1912. The prevention of "woolsorters' disease" (anthrax). *In* Jour. Roy. Sanit. Inst. [London], v. 33, no. 10, p. 507–514.
(3) Gegenbauer, Viktor, and Reichel, Heinrich.
   1913. Die Desinfektion milzbrandiger Häute und Felle in Salzsäure-Kochsalzgemischen. *In* Arch. Hyg., Bd. 78, Heft 1/3, p. 1–128. Literatur, p. 123–126.
(4) Hilgermann, R., and Marmann, J.
   1913. Untersuchungen über die durch Gerbereien verursachten Milzbrandgefahren und ihre Bekämpfung ... *In* Arch. Hyg., Bd. 79, Heft 4/5, p. 168–258. Literaturverzeichnis, p. 256–258.
(5) Hill, H. W.
   1898. A method of preparing test objects for disinfection experiments. *In* Pub. Health Papers and Rpts. Amer. Pub. Health Assoc., v. 24, p. 246–249, 1 pl.
(6) Krönig, B., and Paul, Th.
   1897. Die chemischen Grundlagen der Lehre von der Giftwirkung und Desinfection. *In* Ztschr. Hyg. u. Infektionskrank., Bd. 25, Heft 1, p. 1–112, 2 fig., pl. 1.
(7) Moegle, Erich.
   1912. Zur Desinfektion milzbrandsporenhaltiger Häute und Felle. *In* Centbl. Bakt. [etc.], Abt. 1, Orig., Bd. 66, Heft 5/6, p. 442–462. Literatur, p. 462.

(8) OTSUKI, Ukichi.

    1900. Untersuchungen über den Einfluss der Unterlage auf die Wirksamkeit von Desinfektionsmitteln gegenüber Milzbrandsporen. *In* Hyg. Rundschau, Jahrg. 10, No. 4, p. 153–174.

(9) PONDER, C. W.

    1911. The prevention of anthrax infection due to imported hides and skins. (The Seymour-Jones formic-mercury process.) *In* Lancet, v. 181, no. 4601, p. 1260–1262.

(10) ———

    1911. A Report to the Worshipful Company of Leathersellers on the Incidence of Anthrax amongst Those Engaged in the Hide, Skin, and Leather Industries, with an Inquiry into Certain Measures Aiming at its Prevention. 88 p., illus., 111 diagr. London. Bibliography, p. 69–72.

(11) ROOS, Otto.

    1912. Ueber die Einwirkung von Salvarsan auf Milzbrandbacillen. *In* Ztschr. Immunitätsforsch. u. Exp. Ther., T. 1, Orig., Bd. 15, Heft 6, p. 487–505.

(12) SCHATTENFROH, A.

    1911. Ein unschädliches Desinfektionsverfahren für milzbrandinfizierte Häute und Felle. *In* Wiener Klin. Wchnschr., Bd. 24, No. 21, p. 735–736.

(13) SCHNÜRER, J.

    1911. Zur Frage der Häutedesinfektion. *In* Tierärztl. Zentbl., Jahrg. 34, No. 29, p. 443–444.

(14) ŠEVČÍK, Franz.

    1913. Experimentelle Beiträge zur Frage der Desinfektion milzbrandsporenhaltiger Häute und Felle. *In* Ztschr. Infektionskrank. [etc.], Bd. 13, Heft 6, p. 323–348; Heft 7, p. 439–452.

(15) ———

    1914. Zur Desinfektion von Milzbrandhäuten. *In* Wiener Tierärztl. Monatsschr., Jahrg. 1, Heft 3, p. 127–131.

(16) SEYMOUR-JONES, Alfred.

    1910. The Seymour-Jones Anthrax Sterilization Method. 31 p. London.

OBSERVATIONS ON RHIZINA INFLATA

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Considerable doubt exists regarding the parasitism of *Rhizina inflata* (Schäff.) Sacc. (*R. undulata* Fr.). This peculiar fungus (Pl. VIII, figs. 1, 2, and 3) occurs quite abundantly on the ground in the forest-fire areas of the Northwest. Usually found as a saprophyte on the burned forest soil, it attracted little attention until the close proximity of the fruiting bodies to dead coniferous seedlings was noted to be of frequent occurrence. A close examination of the roots of the dead seedlings showed the mass of white mycelium clinging to and ramifying in the cortical tissues of the root to be in connection with the near-by fruiting structures of *Rhizina inflata*. In some cases the sporophores of this fungus surrounded the stem of the seedling.

The observations on the parasitism of this fungus are not extensive. The disease "la maladie du rond" of *Pinus sylvestris* and *P. maritima*, according to the investigations of Prillieux and De la Boulaye (1880),[1] is accredited to this fungus. Hartig (1891, 1892, 1894, p. 123–129) afterwards in more thorough investigations substantiated the observations of the former investigators and showed *Rhizina inflata* to be capable of living as a true parasite, causing the death of 4-year-old seedlings of *Abies pectinata*, *Pinus strobus*, *Larix europaea*, *Picea sitkaensis*, *Tsuga mertensiana*, *Pseudotsuga douglasii*, and *Castanea vesca*. Von Tubeuf (1897, p. 273) also reports the fungus as a parasite in the forest-tree nurseries of Germany and in the natural forests of *Pinus pinaster* in France.

Early in the spring of 1912 at a certain point along an old logging road in the Kaniksu National Forest, Idaho, where the brush had been burned, young 3- to 5-year-old seedlings of *Tsuga heterophylla*, *Larix occidentalis*, and *Pinus monticola* were observed to be dying in small isolated patches. The roots of the seedlings on being pulled up were closely matted together by a white mycelium, causing a quantity of earth to adhere to them. Since fungous fruiting bodies were not in evidence on any part of the diseased plants or on the ground around, the death of the seedlings was attributed to *Armillaria mellea* (Vahl) Quél., which is very abundant in this region and is frequently the cause of the death of very young growth. The mycelium had penetrated all parts of the cortical and bast tissues of the roots, causing them to become saturated with resin, a condition quite similar to that produced by *A. mellea*. The diseased areas were

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[1] Citations to literature in parentheses refer to "Literature cited," p. 95.

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from 2 to 4 feet in diameter and were irregularly circular in shape, as if the causal agent had started from the center.

Later in the season, near the borders of these areas and at the base of the stems of the dead seedlings, deep-brown, effused, undulating, fruiting structures appeared, which were at once recognized as those of *Rhizina inflata* (Pl. VIII, fig. 2). As to the connection of these fruiting structures with the mycelium beneath them in the forest mold and with that of the roots of the diseased seedlings, there seemed little room for doubt. It did not seem probable that the base of the diseased plant would be completely inclosed by the fruiting structure, with its peculiar rootlike fibrils (Pl. VIII, fig. 3) mingling with the mass of mycelium about the diseased roots, without having some connection with it. Such a seedling with fruiting body attached was carefully removed from the soil and placed in a dish of water, in order to allow the attached earth to fall gradually away. It was found that the numerous rhizoids or strands of mycelium by which the fruiting structures are attached to the substratum were continuous with the mycelium surrounding the diseased roots. These roots were microscopically examined and showed that the internal mycelial system ramifying in the cortical parenchyma and in the sieve tubes of the bast was a continuation of the mycelium which connected up the rhizoid strands of the fruit body.

By shaking in boiled water a quantity of soil which had been burned over the previous year and which showed no signs of fungous growth, a solution was prepared to which a large quantity of spores of *Rhizina inflata* was added. This solution was thoroughly sprayed about the base of several healthy 3- to 4-year-old white-pine seedlings (*Pinus monticola*) growing on burned ground in another part of the forest. The sprayed seedlings appeared slightly reduced in vigor in the fall of 1912 and by July of 1913 they were dead. The roots of each were infected by the same clinging mass of mycelium previously described. The stems and leaves were free from any other diseases. It is believed that this result, although not obtained under control conditions, furnishes some experimental proof of the parasitism of *Rhizina inflata* as it occurs in the Northwest.

Underwood (1896) reports the distribution of the species as follows: Connecticut (Thaxter), New York (Peck), Rhode Island (Bennett), Pennsylvania (Schweinitz), Wisconsin (Bundy), North Carolina and South Carolina (Curtis). The range of *Rhizina inflata* is further extended by the writer, who has collected it at the following stations: Priest River, Idaho, in Kaniksu National Forest on *Pinus monticola*, *Tsuga heterophylla*, and *Larix occidentalis;* Coeur d'Alene National Forest, Idaho, on *Pinus monticola* and *Abies grandis;* Thompson Falls, Mont., in Cabinet National Forest, on *Pinus contorta;* Missoula National Forest, Mont., on *Pinus ponderosa;* Lolo National Forest, Mont., on *Pinus monticola;* Ely, Minn., in Superior National Forest, on *Pinus divaricata;* and Salmon Arm, British Columbia, on *Pseudotsuga taxifolia*.

LITERATURE CITED

HARTIG, Robert.

    1891. Untersuchungen über Rhizina undulata. *In* Bot. Centbl., Bd. 45, No. 18, p. 237–238.

    1892. Rhizina undulata Fr. Der Wurzelschwamm. *In* Forstl. Naturw. Ztschr., Jahrg. 1, Heft 8, p. 291–297, 10 fig.

    1894. Text-Book of the Diseases of Trees. Trans. by William Somerville, rev. and ed. by H. M. Ward. 331 p., 159 fig. London and New York.

PRILLIEUX, E. E., AND BOULAYE, Seurrat de la.

    1880. [Quelques renseignements sur la maladie dite du rond dans les pineraies.] *In* Compt. Rend. Soc. Agr. France, t. 11, p. 386–389.

TUBEUF, Karl von.

    1897. Diseases of Plants Induced by Cryptogamic Parasites . . . Eng. ed. by W. G. Smith. 598 p., 330 fig. London, New York, and Bombay.

UNDERWOOD, L. M.

    1896. On the distribution of the North American Helvellales. *In* Minn. Bot. Studies, v. 1, p. 483–500.

PSEUDOMONAS CITRI, THE CAUSE OF CITRUS CANKER

[A PRELIMINARY REPORT]

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During the summer of 1914 reports of the rapid spread of Citrus canker and the severe injury caused by this new Citrus disease were received by the Bureau of Plant Industry from orange and grapefruit growers in Florida, Texas, and Mississippi. It soon became evident that this disease was one of unusual virulence, which made the investigation of its cause a matter of urgent importance. From the reports of various investigators it appears that Citrus canker was known and recognized as a new disease before any specimens were received by this Bureau.[1] The first specimens received by the Bureau consisted of fruits, leaves, and twigs of grapefruit and showed cankers in every stage of development, from the youngest infections, which were scarcely more than a millimeter in diameter, to the large corky forms, as much as 5 mm. in diameter. A careful microscopic study was made of some of the youngest cankers, and the presence of bacteria was immediately detected. Bacteria were found in fresh sections and have been demonstrated in a large number of stained sections, as represented in the accompanying illustration (Pl. IX, fig. 1).

Numerous plate cultures were made from fresh specimens of cankers received at different times, and an organism was isolated which has been proved to be pathogenic to grapefruit seedlings.

Due attention has been given to all the rules governing bacteriological technique, and every precaution has been observed in making the inoculations. The inoculations were made on young, healthy, vigorously growing grapefruit seedlings, which were kept in the laboratory because the highly infectious nature of the disease made it impossible to carry on the experiments in the Department greenhouses. Pure cultures of the organism were mixed with sterile distilled water, and the suspension thus obtained was placed upon the upper and the under leaf surfaces by means of a sterile pipette in such a manner that the leaves were, for a short time at least, covered with a film of the inoculating fluid. The main stem and branches were treated in the same way. In some cases the leaves and stems were punctured with a sterile needle, but this is not

[1] Stevens, H. E. Citrus canker. A preliminary bulletin. Fla. Agr. Exp. Sta. Bul. 122, p. 113–118, fig. 44–46. Mar., 1914.

Berger, E. W., Stevens, H. E., and Stirling, Frank. Citrus canker. II. Fla. Agr. Exp. Sta. Bul. 124, p. 27–53, fig. 7–14. Oct., 1914.

Edgerton, C. W. Citrus canker. La. Agr. Exp. Sta. Bul. 150, 10 p. Oct., 1914.

Wolf, F. A., and Massey, A. B. Citrus canker. Ala. Agr. Exp. Sta. Circ. 27, p. 97–101, illus. May, 1914.

necessary, as infections may be obtained without this procedure. As soon as the plants were inoculated they were placed under bell jars and kept at a temperature of about 86° F. Under these conditions the organism takes a vigorous hold on its host, and in three or four days evidences of infection can be noted. At the end of a week definite, well-defined cankers which penetrate the tissue of the leaf have been formed. Owing to the stimulating influence which the organism has upon the infected leaf tissue, there is a rapid development of cells, and the tension resulting from the abnormal growth quickly ruptures the epidermis and exposes the soft, spongy, underlying canker tissue, which is distinctly visible on both sides of the leaf. The cankers produced by artificial inoculation present a characteristic appearance and closely resemble natural cankers in macroscopic as well as in microscopic features. They penetrate the tissue of the leaf and are more or less raised on both the upper and the lower surface. The outline is circular, and there is a sharp, distinct demarkation between the canker and the surrounding normal leaf tissue. Young cankers have a soft, spongy structure and at first show a light-green color, which later turns red-brown. The cells in the canker tissue become suberized and produce a corky growth, which is a symptom of the disease. This open, spongy type of canker is the result of rapid growth due to favorable conditions of temperature and moisture.

The identity of natural and artificial cankers is shown in Plate X. Sections of cankers about 2 weeks old show the pathological and histological features observed in young natural infections. (See Pl. IX, fig. 1.) The cells are found to be filled with short rod bacteria, and the stimulus exerted by the organism on the infected tissue is distinctly visible. The natural differentiation of palisade and parenchyma tissue has been obliterated, and all the cells exhibit more or less enlargement and distortion, which is due to the activity of the invading organism. As a result the diseased tissue of the canker is raised above the normal leaf surface. In later stages in the development of the canker some of the cells disintegrate, and lesions are formed. The organism appears to act more vigorously on the cell contents than on the cell walls, and in due time the cell contents are exhausted. The cell walls which remain become suberized and constitute the corky cankerous growth which is a characteristic symptom of this disease. Numerous cankers obtained from pure-culture inoculations upon grapefruit seedlings are shown in Plate IX, figures 3, 4, 5, 6.

While the canker is still soft and young, the organism is in a very active condition and can be isolated very readily. Upon teasing out a small piece of canker tissue in a drop of sterile water, motile bacteria in great numbers ooze out and give the water a milky, turbid appearance. The motility of the organism can be most satisfactorily observed by means of dark-field illumination. The organism was reisolated from

these cankers by plating out on beef agar and was found to be identical with the original organism. Inoculations on grapefruit plants with the organism obtained from this reisolation produced characteristic cankers.

The open surface of the canker and the spongy character of its structure afford an excellent lodging place for spores of all sorts, and it is not surprising to find fungi, some of which may perhaps play a minor part in the later stages of the disease. A number of fungi have been isolated from old Citrus cankers, and a study of their relation to the canker problem shows that the fungous flora of the Citrus canker perhaps may be an interesting problem in itself.

The organism appears to be a new species and is briefly described as follows:

Pseudomonas citri, n. sp.

This organism is a short, motile rod with rounded ends and a polar flagellum. It occurs singly or in pairs and varies in shape from a short, ellipsoidal form to the typical rod. Its dimensions show corresponding differences, but rod forms usually are 1.5 to 2 by 0.5 to 0.75μ.

When plated out on beef agar at room temperature, the organism appears at the end of 36 to 48 hours, the colonies showing up as fine, glistening points just visible to the naked eye. The surface colonies increase quite rapidly in size and in three or four days show very distinctly. They are circular in outline, with entire margins and a slightly raised, smooth surface. By reflected light the colonies show a dull yellowish color, while a bluish translucent color is observed by transmitted light. The internal structure is finely granular and the motility of the organism can sometimes be noted in the outer border of the colony by examining the culture under the low power of the microscope.

In needle-stroke cultures on beef agar a moderate filiform growth is produced which does not penetrate the agar. The streak widens slowly and spreads more at the base of the slant surface. The bacterial mass is slightly raised, smooth, shining, and dull yellow in color.

A very characteristic growth is obtained on potato cylinders. In young cultures the organism follows the line of the streak and produces a somewhat raised, shining growth which has a bright-yellow color. A narrow, white zone is noted on the uninfected surface of the potato, following the margin of the bacterial mass. This feature does not persist very long, as the organism grows vigorously on this medium and soon the entire surface of the cylinder is covered with a thick, yellow, shining, viscid mass.

Beef bouillon shows a visible growth in 24 hours. In older cultures a yellow ring is formed at the surface.

Litmus milk shows a deeper blue color, the casein is precipitated, and the clear supernatant liquid appears a deep reddish color when viewed by transmitted light.

Gelatin is liquefied, the line of puncture is filiform, and the growth of the organism takes place at the surface of the culture.

Dunham's solution shows more or less clouding, the heaviest growth taking place in the open end of the tube, where a flocculent growth is noted at the surface. No traces of indol were noted.

This organism produces no gas in the presence of Dunham's solution in combination with dextrose, lactose, or mannit. The organism grows well in all these combinations, especially at the open end of the tube, where a flocculent growth is produced. Dextrose appears to favor the development of this organism particularly, as a heavy, flocculent growth is formed throughout the entire tube. It grows but sparingly in Ushinsky's solution, and in starch-nitrate solution does not reduce the nitrate. The organism grows best under aerobic conditions.

The organism stains readily with carbol fuchsin, and flagella have been demonstrated by means of the methods of Van Ermengem and Dr. Hugh Williams. (See Pl. IX, fig. 2.)

Much confusion and uncertainty seem to exist in the minds of Citrus growers and others in regard to the identification of the true Citrus canker. Many specimens supposed to be infected with canker which have been sent for identification have been found to be injured by fungi or some other cause. A most careful and detailed comparative study of Citrus canker and other diseases resembling it must be made in order to clear up the canker problem and reduce the necessity of frequent bacteriological diagnoses.

Although this paper gives only a very brief account of the etiology of the Citrus canker and many important facts in the life history of the causal organism remain to be determined, the immediate publication of this preliminary report is considered necessary on account of the great economic significance of this disease, which up to the present has been supposed to be due to a fungous parasite. Because the methods of control for bacterial diseases differ quite radically from those employed for fungous diseases it is hoped that the presentation of this report at this early stage in the investigation will lead to a more adequate understanding of the precautions which may be essential in an effective campaign of eradication.

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PLATE IX

Pseudomonas citri

Fig. 1.—Drawing of a stained section of a portion of a grapefruit leaf bearing a young canker resulting from inoculation with a pure culture of *P. citri.* ✕ 250.

Fig. 2.—Photomicrograph of *P. citri* stained by the Williams method for flagella. ✕ 1,000.

Fig. 3.—Top view of a grapefruit seedling showing the results of artificial inoculation with *P. citri* isolated from Texas specimens.

Fig. 4.—View of the lower side of the leaves shown in figure 3.

Fig. 5.—Top view of a grapefruit seedling showing the results of inoculation with *P. citri* obtained from Florida specimens.

Fig. 6.—View of the lower side of the leaves shown in figure 5.
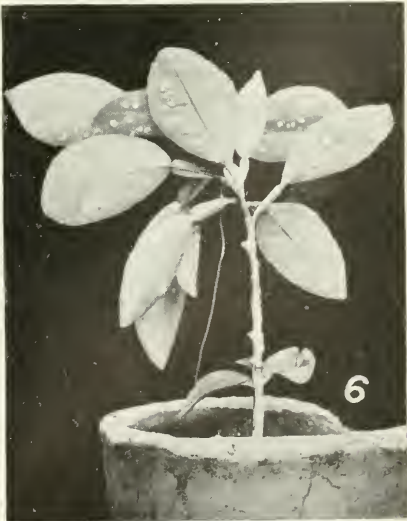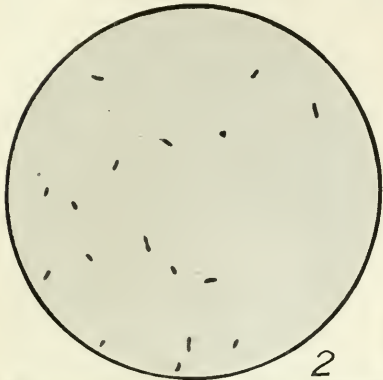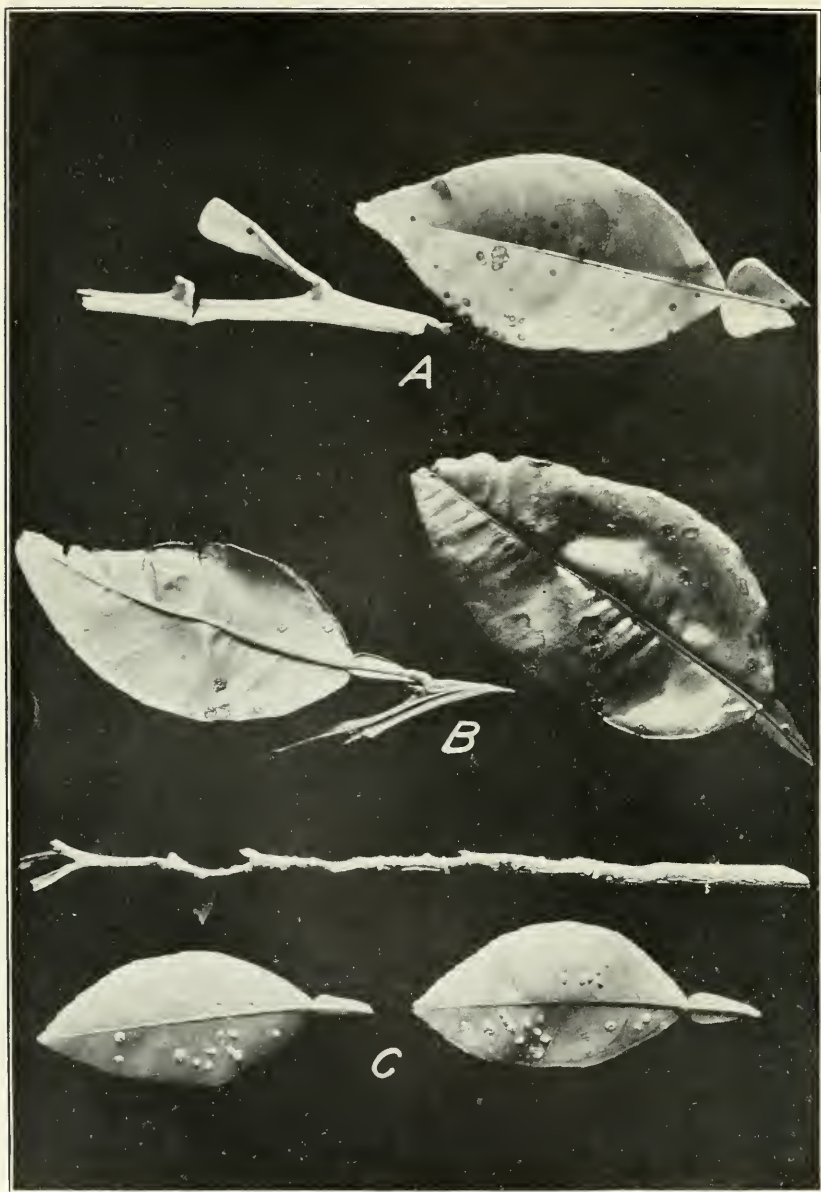
PLATE X

PLATE X

Pseudomonas citri: Small lesions on Citrus twigs and more obvious cankers on Citrus leaves. *A*, Cankers on twig and leaves from Florida produced by natural infection; *B*, natural infections on leaves from Texas; *C*, cankers on twig and leaves produced by artificial inoculation.

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WILT OF GIPSY-MOTH CATERPILLARS [1]

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INTRODUCTION

The present investigation of the wilt of gipsy-moth caterpillars (*Porthetria dispar* L.) was undertaken in the hope of obtaining results of economic importance. Two questions that have caused considerable speculation are, When did wilt first appear in the United States? and How did it get here? The gipsy moth was brought from France to Medford, Mass., in 1869, but it did not become a very serious pest before 1889, when active suppression work was begun by the State of Massachusetts. However, there is no account of the appearance of wilt prior to 1900, although old State records and documents have been gone over very thoroughly. The writer has talked to a number of people, but no one seems to have seen wilt before 1900. Among these was Mr. C. W. Minott, who has had much experience with the gipsy moth since 1894. In response to an inquiry of the writer in regard to the history of wilt, Prof. Charles H. Fernald, of the Massachusetts Agricultural College and formerly entomologist of the Massachusetts State Board of Agriculture, wrote as follows:

From the first noticeable outbreak of the gipsy moths in Medford in 1889 up to 1900, when the legislature closed the work, I was in close touch with it and spent all the time I could spare from my college work here. During that time I neither saw nor heard of the "wilt" disease nor of anything in any way resembling it. I went down there every week and was around with the men in the field in every part of the infested region, and if either they or the field director had noticed anything of the kind they would surely have told me, for they all knew that we were hunting for and breeding all the parasites we could find.

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How the wilt of gipsy-moth caterpillars was brought to this country will probably always remain a puzzle, but many possibilities suggest themselves. Wilt and *Wipfelkrankheit*, a disease of the European nun-moth caterpillars, may be identical, and the disease may have been intro-duced on trees and shrubbery or other material. This is not at all improbable, for when caterpillars die and disintegrate on trees, the virus may dry on them, making it easy for the disease to gain entrance into this country on shipments of plants. This seems very likely in the light of recent investigations by Escherich and Miyajima (4) and Prowazek (12) on the long resistance to drying of the virus of Wipfelkrankheit and Gelbsucht.

Then, again, wilt may have been introduced from its original source with the parasites in 1905, when the State of Massachusetts, in coop-eration with the Federal Bureau of Entomology, imported large num-bers of parasites and natural enemies of the gipsy moth from its native homes in Europe and Japan. The first definite printed record of the wilt disease is the one given two years later by Howard and Fiske (9). One of the tachinid flies, *Compsilura concinnata* Meig., in the vari-ous stages of its life history is especially well adapted to aid in the rapid dispersion of the disease. This imported parasite and others which are spreading rapidly may be the cause of the prodigious increase in wilt mortality since 1907.

Finally, the wilt of the tent caterpillar and that of the gipsy moth may possibly be identical, and the latter, though not previously affected, for some reason may have become susceptible to the disease of the former. This last theory does not seem very plausible, however, and ought not to be considered seriously till we have experimental proof of the identity of the wilt diseases of the tent caterpillar and gipsy moth.

DISTRIBUTION AND EPIDEMIOLOGY OF WILT

There is every reason to suppose that the wilt is distributed over the entire territory infested by the gipsy moth. In the summer of 1913 the writer personally visited places in Maine, New Hampshire, Massachusetts, and Rhode Island during the caterpillar season and found the disease to a greater or less extent in all these States. To be absolutely certain, material was always collected from the points visited and was later examined microscopically for polyhedra (p. 104). The field men of the Bureau of Entomology scattered throughout the area of infesta-tion sent in diseased material from localities which the writer was unable to visit. In this way records of some 112 separate localities where the disease occurred, including the writer's observations, were obtained.

At present the gipsy-moth-infested area in Maine embraces about 4,850 square miles; in New Hampshire, 4,960 square miles; in Massa-chusetts, 4,975 square miles; and in Rhode Island, 450 square miles. Wilt, based on microscopical examinations, was found in 4 places

visited in Maine, 15 in New Hampshire, 90 in Massachusetts, and 3 in Rhode Island. That the number of infested places in Massachusetts given above exceeds those of New Hampshire is probably owing to the fact that 75 more places were studied in the former than in the latter State and that the number in New Hampshire exceeds that in Maine is due to a similar reason, for 11 more localities were visited in New Hampshire than in Maine. In short, the disease was found wherever close and continuous observations were made, with the possible exception of one or two places, but even these were doubtfully healthy.

The epidemiology of wilt is not noticeably different from that of Wipfelkrankheit, for the writer was able to confirm most of the field observations of Wahl (18), Tubeuf (14, 15), and others who have studied the nun-moth disease. When a territory becomes heavily infested, an epidemic occurs sooner or later, for these larvæ defoliate all the trees and later many congregate in masses on the trunks. Naturally when the disease breaks out in such a mass most of the caterpillars become infected, and since they are everywhere abundant and are crawling around in search of food, infected individuals rapidly spread the disease. The lack of food, which is necessarily brought on by defoliation, furthermore, causes caterpillars to lose their vitality, producing greater susceptibility to the disease. Gipsy-moth caterpillars mature in July, when it is usually very hot, and after having stripped a tract of woodland of its leaves, they are almost entirely exposed to the sun's rays. Escherich and Miyajima (4) have shown experimentally that sunlight can convert the chronic into the acute form of wilt, and one can readily become convinced of the accuracy of this observation by visiting a heavily infested, stripped piece of woodland during a hot spell. Thousands of gipsy-moth caterpillars that have died of this disease will be found hanging to limbs and tree trunks (Pl. XI). There will be an enormous reduction in the number of adult moths and consequently in the number of egg clusters, but a complete extermination does not take place, owing in part to the immunity of certain individuals (p. 124).

In a lightly infested woodland the conditions are different. Here the caterpillars are much more widely separated and an epidemic is not produced. There is sufficient food throughout the season, and the trees are never completely defoliated; hence, caterpillars can always find cool places in which to rest during the midday heat. Yet even in such a favorable locality a few caterpillars will die, but the mortality as a whole is small in proportion to the number of individuals; many apparently escape infection, and most of the next generation will escape likewise the following year, unless the increase of caterpillars produces an epidemic.

Wilt is more prevalent among the older caterpillars for the reasons given above, but younger caterpillars also die of the disease, as is shown by field observations during the season of 1913, when a few typical cases were found as early as May 27, at which time the temperature

ranged between 51° and 69° F. Of course, it has been known for a long time that first and second stage caterpillars will die of the wilt when kept in a warm laboratory under unfavorable conditions, but the foregoing observations demonstrated that sometimes these caterpillars will succumb in the field even under what seem to be most favorable conditions. Many small caterpillars die of starvation, especially on coniferous trees, and many undoubtedly meet death after exposure on a cold night; therefore no diagnosis will be valid unless made microscopically for the polyhedra described below.

In some localities studied during the summer of 1913 wilt did not appear until very late in the season, when most of the caterpillars were full grown and pupating. In an infestation near Provincetown on Cape Cod, Mass., no indications of the disease were found till the very last of July, when the caterpillars were beginning to pupate. The colonies at Provincetown are isolated from the remaining infestations in Massachusetts and it seemed likely that the disease had not spread to this locality, but visits during the latter part of the season demonstrated the existence of true cases of wilt. Provincetown faces the Atlantic, and the rather cool climate may have kept diseased individuals in a chronic condition for a long time. That is the reason wilt was not noticed earlier. This example is cited simply to show how careful one must be in pronouncing a locality healthy.

PATHOLOGY OF WILT

When a caterpillar dies of wilt, all of its tissues are in a state of disorganization. The intestine is the last internal organ to disintegrate, owing to the fact, perhaps, that it is capable of resisting the fermentative or toxic character of the virus longer than the remaining tissues. If a smear of the brown liquid from a dead caterpillar is examined microscopically with a high-power dry or oil-immersion lens, it will be found to contain, besides the elements of disorganized tissues, myriads of polyhedral bodies of various sizes. (Pl. XII, fig. 1, and text fig. 1.) The average polyhedron measures from 1 to 6μ in diameter, and the individual faces of such a single body vary also. Certain polyhedra have been found to measure $\frac{1}{2}\mu$ and less, while still others reach the size of 15μ. If there is plenty of liquid on the slide, air currents will cause the polyhedral bodies to turn over and over, so that one can obtain excellent views of all their faces. Their shape varies as much as their size, but in general the form is that of a polyhedron, with more or less rounded angles. They never assume the shape of a perfect sphere, and an actual geometric outline has never been observed, as is the case with the silkworm polyhedra, which are almost perfect octahedra. (Pl. XIII, fig. 1.) In general appearance the polyhedra of the gipsy moth are more like those of the nun moth than those of the silkworm.

The wilt polyhedra are highly refractive, and on focusing they are seen to have a denser center differentiated from a somewhat lighter peripheral mass. Sometimes within the bodies concentric layers like those of an onion are observable. Often two polyhedra are seen adhering to one another, as if in the act of dividing (Pl. XIII, fig. 2), but an actual division in a hanging-drop has never been noticed, although several preparations were kept upon the microscope stage under continual observation for more than six hours. When pressure is applied to the cover glass, the polyhedra crack very readily into a number of pieces (Pl. XIII, fig. 3–10), and often without the application of pressure the same fragmentation may be observed to occur somewhat more slowly. In the latter case a notch appears at one side of the polyhedron, which gradually lengthens into a line progressing slowly toward the other side, much like the cracking of ice. Usually before the line has completely separated the two halves other lines appear, and soon the entire polyhedron is divided into a number of pieces, which may separate or may stick together in a rosette-like fashion. At no time was anything observed to come out of the polyhedra when they cracked in this manner. If the cover glass is moved while applying a little pressure, one half of the polyhedron may sometimes be folded upon the other half without the cracks appearing, showing that it is

Fig. 1.—Drawing of polyhedral bodies as seen in smears of "wilted" caterpillars.

composed of a tough substance and is not at all brittle, like inorganic crystals.

The only objects in a fresh preparation with which one could possibly confuse the polyhedra are the fat globules and urate crystals, but with a little practice these may be readily distinguished. Fat globules are perfectly spherical and are therefore unlike the polyhedral shape of the bodies in question; but, when in doubt, Sudan III was used, for in this stain the fat globules become red, while the polyhedra remain colorless. The urate crystals are often more acutely angular or are of an entirely different shape from the polyhedra and are frequently traversed by radiating lines (Pl. XIII, fig. 11–18).

Besides polyhedral bodies, fat globules, and urates, a smear from a newly "wilted" caterpillar contains cellular débris, hairs, and pigment granules. The pigment granules must not be confused with bacteria,

for many of them superficially resemble these organisms very closely. When a preparation is dried, mounted, and examined under oil, the pigment granules of the gipsy moth may easily be confused with micrococci, owing to the fact that they are usually arrayed in pairs. As a matter of fact, a smear made from a recently wilted caterpillar is almost devoid of bacteria, and in many cases none at all can be found. If bacteria are found, they have escaped into the body cavity through rupture of the intestine and bear no direct etiological relation to the disease, as will be shown later. The very minute, double, dancing granules in a fresh smear, apparently neither pigment nor bacteria, will be treated in more detail in later pages.

In fixed and stained smears a number of things can be demonstrated to advantage within the polyhedra. Fixation was accomplished either by passing the preparation through a flame or by placing it in absolute alcohol for a few minutes. The smears were then stained in Giemsa's solution for 12 hours or were stained for a shorter period with one of the following dyes: Methylene blue, trypan blue, gentian violet, carbol fuchsin, Bismarck brown, or iron hæmatoxylin. When iron hæmatoxylin was used, the preparation was first mordanted in a 4 per cent ferric-alum solution for two or three hours. Gram's method of staining, Moeller's spore stain, and Welch's capsule stain were also tried, but these were of no greater advantage than the simpler ones given above. After staining, the preparations were sometimes quickly passed through the alcohols to xylol before mounting. This not only clears everything, but dissolves away all the fat, thus increasing the transparency of the preparation. The polyhedra are very resistant to stains in general and usually color along the periphery only, unless the stain is applied for a long time. On so doing one can succeed in staining the entire polyhedron, especially after the use of some mordant like ferric alum before hæmatoxylin or anilin water before gentian violet. Steaming the preparation with a stain like carbol fuchsin has also given good results.

When properly stained, one of three conditions is obtained: First, the polyhedral bodies are uniformly stained so that nothing can be detected within them; or, second, a uniformly darker staining central mass can easily be differentiated from an almost unstained outer substance (Pl. XIII, fig. 19); or, third, many little refractive, reddish granules are seen within the polyhedra (Pl. XIII, fig. 20). I have obtained very good preparations of these three conditions, especially by the use of iron hæmatoxylin, methylene blue, and gentian violet. An actual differentiation between what might be interpreted as nuclear and cytoplasmic material within the polyhedra never occurs; therefore, in accounting for the staining reactions the writer believes that at times the polyhedra have a central granular or homogeneous substance easily distinguishable from an outer substance which is more resistant to the dyes. This varies a great deal, however, for very often the periphery of the

polyhedra takes the stain more readily than the underlying strata. From these staining reactions it becomes apparent that the polyhedra of the gipsy moth are complicated in structure, thus not differing essentially from what Bolle (1) and Prowazek (12, p. 277–281) found to be true of the silkworm polyhedra.

The polyhedra are heavier than water and consequently can be obtained in bulk by centrifuging aqueous emulsions of diseased material. By repeated washing and centrifuging, most of the fat, the cellular débris, etc., can be eliminated and the polyhedra obtained in a fairly pure stage for chemical tests. The writer has found, as did Prowazek (11) that 1 per cent of sodium hydroxid or potassium hydroxid swells the polyhedra to about double their normal size and that the same granular mass observed above again becomes visible. After a time the granular mass flows together and disappears, a sort of shadow remaining. On treating the material with dilute hydrochloric or sulphuric acid, the granular mass reappears and flows together; then the periphery of the polyhedron dissolves away, and a shadow remains, which also quickly vanishes. The wilt polyhedra do not dissolve in hot or cold water, and are insoluble in alcohol, ether, chloroform, xylol, and glycerin, but are soluble in strong acids and alkalies. They do not blacken with osmic acid and do not stain with Sudan III and therefore contain no fat. Picric acid stains them yellow, showing that they are related to the albuminoid substances.

So far nothing tangible has appeared that would enable the writer to regard the polyhedral bodies as organisms, and therefore he believes them to be reaction bodies belonging to the highly differentiated albumins— namely, the nucleoproteids. It will be shown later that the polyhedra originate in the tissue nuclei; hence, the conception of nucleoproteid reaction bodies does not seem unjustifiable. Furthermore, experiments discussed later in this article show that it is possible to infect caterpillars with material from which the polyhedra have been removed.

Although the writer has examined thousands of stained smears of wilted caterpillars, he has never observed anything which could be associated with the chlamydozoa as described in 1907 by Prowazek (11). In his latest paper, however, Prowazek (12) himself says very little about the chlamydozoa and therefore seems no longer to be greatly impressed with their etiological importance.

TECHNIQUE

Before going into a detailed consideration of the pathology of the tissues, a description of a number of the fixing and staining methods that were used may not be out of place.

The best results were obtained by the use of Giemsa's stain, Schaudinn's corrosive-sublimate fixation being used whenever this stain was employed. Hot water was saturated with corrosive sublimate and allowed to cool. Two parts of this solution to one part of absolute alcohol constituted the fixing fluid, which was used cold. The

caterpillars, if small, were either punctured or split open dorsally before being put into the solution or, if large, were cut crosswise into three or four pieces and allowed to fix for 48 hours. After using the fixing fluid, the caterpillars were immersed in 95 per cent alcohol, then in 100 per cent alcohol, and next in cedar oil, in which the material was cleared for 48 hours. The specimens were then embedded in paraffin, and sections were cut of the thickness of 2, 3, 4, or 5μ.

The process of staining and differentiation used is based upon Wolbach's modification of Giemsa's method.[1] The various processes to which the cut sections were subjected according to this method can be followed best in a numerical series.

(1) Xylol; (2) absolute alcohol; (3) 95 per cent alcohol; (4) iodin alcohol (100 c. c. of 70 per cent alcohol plus 3 or 4 c. c. of a saturated alcoholic solution of iodin); (5) 95 per cent alcohol; (6) distilled water; (7) hyposulphite of soda (0.5 per cent) in distilled water; (8) washing sections in running tap water for five minutes; (9) rinsing in distilled water; (10) staining with Giemsa's solution, changing stain twice at one-half hour intervals, leaving sections in third solution overnight (about 12 to 15 hours); (11) acetone-colophonium mixture for about one minute (this differentiates and consists of 30 gm. of colophonium to 200 c. c. of acetone); (12) acetone-xylol mixture, which stops the destaining and consists of 70 c. c. of xylol and 30 c. c. of acetone; (13) xylol; (14) mounting in thick cedar oil.

The Giemsa's solution was made from the stains of Grübler's manufacture. At first the mixture was bought all ready "made up" by local chemists, but the results were unsatisfactory and too variable to be depended upon. It was not until the writer made his own mixture that Giemsa's stain was a complete success.

The stock solution is made up as follows:

Azur II eosin...	3 gm.
Azur II..	0.8 gm.
Methyl alcohol (c. p.).......................................	375 gm.
Glycerin (c. p.; Merck's, sp. gr., 1.250)....................	125 gm.

The staining solution is made when needed from the stock solution, as follows:

Distilled water...	100 c. c.
Methyl alcohol..	4 c. c.

Stain, 40 drops from an eye dropper; 0.5 per cent sodium carbonate, 2 drops from an eye dropper.

Another stain following the corrosive-sublimate fixation and giving very good results is Unna's polychrome blue, which consists of a 1 per cent aqueous solution of methylene blue to which has been added 1 gm. of sodium carbonate. This is allowed "to ripen" for one week. Following are the steps in the staining process to which the sections are subjected:

(1) 5 per cent aqueous w. g. eosin for 20 minutes; (2) Unna's polychrome blue 10 c. c. and 100 c. c. of water till the sections are a deep blue; (3) tap water; (4) differentiation in 95 per cent alcohol containing 10 per cent of resin; (5) absolute alcohol, xylol, Canada balsam.

Some good slides were obtained by fixing with Kahle's fluid and by staining with iron hæmatoxylin and orange G: but this method was not as satisfactory as another one suggested by Prof. Gary N. Calkins, in which the material is fixed for an hour in a fluid consisting of 20 per cent of glacial acetic acid and 80 per cent of saturated aqueous corrosive sublimate. The sections are mordanted for 12 hours in a 4 per cent solution of ferric alum, after which they are stained in a 0.5 per cent solution of aqueous iron hæmatoxylin for 12 to 24 hours. No counterstain is used, for by differentiating with the ferric alum the staining can be stopped at a point where both nucleus and cytoplasm are nicely colored.

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[1] Wolbach, S. B. The filterable viruses. A summary. *In* Jour. Med. Research, v. 27 (n. s. v. 22), no. 1, p. 1-25, 1 fig.

PATHOLOGY OF THE TISSUES

As stated previously, owing to the fact that dead caterpillars disintegrate completely, they can not be used for sectioning; so one has to rely entirely on living, diseased material. By sectioning large numbers of caterpillars or by infecting a number of individuals and sectioning one every few days, all stages of the disease can be obtained. The writer has sectioned between 600 and 700 individuals in all stages of development, from the fully formed embryo within the egg up to the pupa. Polyhedral bodies have never been found in gipsy-moth eggs, although both apparently normal eggs and eggs that did not hatch were carefully examined. The pathological conditions in the post-embryonic stages—i. e., from the first to the sixth or seventh instars—were found to be exactly alike, showing that the pathology does not vary at different ages.

If the anterior and posterior ends of an infected caterpillar be cut off so that the alimentary canal can be pulled out easily, one will find on examination under a low-power microscope that the trachea and its finer branches have grapelike clusters of rounded bodies attached to them. Upon examination under the high-power dry or oil-immersion lens, however, one finds that the clusters are simply masses of polyhedral bodies within the nuclei of the tracheal matrix cells. (Pl. XII, fig. 2.) The nuclei of these cells seem to be among the first to be affected, for often one will have no difficulty in finding polyhedral bodies around the tracheæ, when none will be revealed by a careful search in the other tissues. Later, the polyhedral bodies appear also within the nuclei of the hypodermal, fat, and blood cells. If the pathological nuclei in their earlier stages—i. e., before the polyhedral bodies have reached their final stage of development—are carefully examined under oil, many minute violently dancing granules will be found within them. The dancing granules may be particles of degenerated chromatic or achromatic substance, but the activities are so violent even in a preparation from which all air currents have been excluded with vaseline that the writer is inclined to think that there was more than molecular motion and that he was confronted with the behavior of extremely minute micro-organisms. These granules are similar to those found in the fresh smears of dead caterpillars mentioned previously, in distinguishing between pigment and other more violently dancing particles. For reference to these bodies in stained sections, see page 110.

Stained sections show that the polyhedra originate within the nuclei of the hypodermal, fat, tracheal matrix, and blood cells. The writer has been utterly unable to find polyhedra within the nuclei of the muscles, Malpighian tubes, ganglia, or nerves. It is also interesting to note that polyhedral bodies have never been found within the nuclei of gland cells, such as setiferous cells, intestinal epithelial cells, œnocytes, salivary glands, and gonads.

The formation of the polyhedral bodies within the nuclei of the four tissues above mentioned and the visible changes taking place within these nuclei may be described as follows: The first indication of a diseased nucleus seems to consist in the flowing together of the chromatin into a lump in the middle (Pl. XIII, fig. 21). Then out of the achromatic substance the polyhedra arise as very minute individuals (Pl. XIII, fig. 22), which can be demonstrated to advantage by the hæmatoxylin method given under "Technique." By this method the polyhedra are stained dark; by the use of Giemsa's stain they are merely faintly outlined (Pl. XIII, fig. 23). At this stage Giemsa's stain also clearly shows many little granules in the nuclei (Pl. XIII, fig. 23) which are identical with the dancing granules observed in fresh preparations. They stain red and are either single or double, thus resembling tiny micrococi. These granules may adhere to the periphery of the polyhedra or may lie above, below, or in the spaces between them. The formative polyhedra themselves stain slightly along the periphery with Giemsa's stain. As the polyhedra increase in size, they become more and more refractive, do not stain at all finally, and the nucleus swells to an enormous size (Pl. XII, fig. 3; XIII, fig. 24). To obtain some idea of the comparative sizes of normal and pathological nuclei in the same tissue of the caterpillar, 12 normal and 12 pathological fat cell nuclei were measured. The normal nuclei measured between 6 and 13μ, the pathological nuclei from 7 to 29μ in diameter. The early pathological stages measured less than the later ones, and it is seen from the measurements that the late stages of the hypertrophied nuclei are more than twice as large as the largest normal nucleus. This swelling of the nucleus is due to the increase in size of the polyhedral bodies, which stretch the nuclear membrane. All the polyhedra seem to be in the same stage of development within an individual nucleus—that is, great differences in sizes between polyhedra within a single nucleus do not occur, but there are, of course, enormous variations in sizes between those of separate nuclei. The small polyhedra are somewhat rounder than larger individuals, which can be accounted for by the fact that, as the polyhedra grow, they become so closely packed within a nucleus that they press upon one another and thus the more or less polygonal shape is produced. As the polyhedra grow and become more refractive, the little red granules stained by Giemsa's stain, as well as the remains of the chromatin lump, disappear and there remains simply the nuclear membrane inclosing the polyhedra (Pl. XIII, fig. 24). Sometimes the chromatin lump remains till the nucleus disintegrates, but most frequently it disappears before this event. The nucleus swells more and more, finally the nuclear membrane ruptures, and the polyhedra escape into the body cavity (Pl. XIII, fig. 25). Thus, the polyhedra are found free in great numbers in smears of dead caterpillars.

Although these bodies are not formed within the nuclei of muscle, nerve, excretory, and glandular cells, it is not the intention to imply that no changes at all take place within these, for such is not the case. Their chromatin shows signs of degeneration, such as the flowing together into lumps, but the little reddish-staining granules were never found within them. This leads the writer to believe that the little granules are not products of nuclear disintegration—if they were, one would expect to find them within these nuclei also—but that they are of etiological significance. While, of course, they may be the vegetative stages and the polyhedra the resting stages of an unknown organism, there is nothing tangible which would substantiate the view that the polyhedra are directly related to these granules. The latter are not identical with those appearances described as being within the polyhedra (p. 106). It will be shown later in this paper that the virus passes through the pores of the Berkefeld filter, and since such a filter holds back polyhedra one might say that these have been satisfactorily eliminated and therefore are of no etiological significance; but this, it seems to the writer, is a narrow view to take of the subject. The Berkefeld filtrate revealed little dancing granules which may be identical with those observed within the tissue nuclei. Now, as before stated, these filterable granules may be the vegetative stages and the polyhedral bodies the resting stages of an organism; or the polyhedra may be a secretion of a minute organism contained within. As long as there is no evidence, however, that the polyhedral bodies are directly related to the filterable virus or to the little granules, the view that they are reaction products appeals more strongly. The virus invades the nuclei of the hypodermal, fat, tracheal matrix, and blood cells, and the polyhedral bodies arise, perhaps, as by-products of nuclear digestion and disintegration. When these four tissues disintegrate, it is an easy matter to conceive the disorganization of the remaining tissues, and, as stated previously, the intestine seems to be one of the last organs in the body to be so affected.

The questionable little granules should not be confused with the pigment granules occurring in the hypodermal cells and in the ganglia. Since the pigment granules are larger and are never found within the nuclei, unless carried there by the microtome knife, they are very easily distinguished. Furthermore, the ordinary protein bodies often occurring in the spaces of the fat body and easily demonstrated by hæmatoxylin must not be taken for polyhedral bodies. Protein bodies stain perfectly black with hæmatoxylin, are of a round or a regular shape, and are never found within the nuclei.

Polyhedra have never been found in the intestinal lumen. One would often expect to find them there, especially after artificially feeding polyhedral material to caterpillars, but this does not prove to be the case. However, their absence may be explained by the observations of

Bolle [1] and Prowazek (11). Prowazek found that pepsin hydrochloric acid dissolves the polyhedra of the silkworm, and Bolle found that the juices of silkworm intestines dissolve them likewise, so that it is not at all improbable that the gipsy-moth caterpillars digest polyhedral bodies very rapidly.

The only things found within the intestinal lumen of caterpillars are partly undigested leaf cells and occasional bacteria. The latter are sometimes found in great numbers in the intestines of caterpillars raised on abnormal food in the laboratory, but they are scarcely ever found in sections through diseased individuals taken in the field. Lettuce was frequently fed to caterpillars hatched in the laboratory in the winter, but this is unfavorable food, as cultures showed, being full of bacteria of all sorts, especially when after standing it begins to ferment. That bacteria are not etiologically related to wilt will be shown more definitely in this paper.

PATHOLOGY OF THE BLOOD

Before considering the blood of diseased gipsy-moth caterpillars, the various elements in the hæmolymph of normal individuals should be carefully distinguished. For purposes of microscopical examination a drop of blood can be best obtained by pricking one of the caterpillar's prolegs with a fine needle. In healthy animals the blood is clear; light yellow in males and greenish in females.[2]

The morphological elements or blood corpuscles are represented by two main types. Those of the first type are the ordinary round or amœboid cells, amœbocytes (Pl. XIV, fig. 1, 2). An actual pseudopod-like streaming has never been observed, but, since we find such forms as shown in Plate XIV, figure 1, with foreign bodies within them (phagocytosis), there can be little doubt as to their mode of progression. Graber's view (8) that the form of the leucocyte is due as much to the various blood sinuses as to its own individuality is not held by the writer. Graber says that the blood corpuscles are elastic, but that on their squeezing through narrow passages or sinuses this elasticity is broken down—that is, the corpuscles reach their elastic limit and are unable to reassume their natural sphericity. Hence, the various amœboid and stellate cells are due to the shape and width of the passages traversed. It is, however, generally accepted that the blood corpuscles in most insects move in an amœboid manner, thus resembling the leucocytes of man.

All of the blood corpuscles of insects possess a nucleus, and the leucocytes of the gipsy moth are no exception to this rule. The nucleus is

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[1] Cited by Prowazek (11).

[2] For these interesting sexual differences in the color of caterpillar blood see the following papers:

Geyer, Kurt. Untersuchungen über die chemische Zusammensetzung der Insektenhämolymphe und ihre Bedeutung für die geschlechtliche Differenzierung. *In* Ztschr. Wiss. Zool., Bd. 105, Heft 3, p. 349-499, fig. 58, pl. 20-22. Literaturverzeichniss, p. 488-499. 1913.

Steche, O. Beobachtungen über Geschlechtsunterschiede der Hämolymph von Insektenlarven. *In* Verhandl. Deut. Zool. Gesell., Bd. 22, p. 272-281. 1912.

difficult to see in fresh preparations; but, if a little acetic acid is added or if the corpuscles are properly fixed and stained, it can be very easily demonstrated.

To the second type of corpuscle belong curious corpuscles filled with thick colorless globules (Pl. XIV, fig. 3, 4). This type is not so plentiful as the amœboid, but one often finds two or three of them to a single field. They are nearly always spherical and never emit pseudopodia. At first the writer confused these corpuscles with the pathological forms, for the colorless globules within resemble polyhedra very much at times. They are perfectly normal appearances, however, and are similar to the "mulberry corpuscles" of Forbes (5) and to the corpuscles described by Cuénot in 1891 (2). Cuénot says, in his work on the grass egger (*Gastropacha trifolii*) that the globules within the "mulberry corpuscles" color yellow with iodin and present all the various albumin reactions. He further states that they are reserve amœbocytes and that various transitional stages between them and the ordinary amœbocytes, in which the albumin (protein) globules accumulate gradually, can be detected. The "mulberry corpuscles" likewise possess a nucleus, which is surrounded by the protein bodies. This nucleus can be very easily seen by crushing the corpuscle (Pl. XIV, fig. 5).

When a gipsy-moth caterpillar becomes infected with wilt, polyhedral bodies begin to form within the nuclei of the amœbocytes in a manner very similar to their method of formation within the nuclei of the tissue cells previously described. When the polyhedra originate within the nuclei of the blood corpuscles, they have the appearance shown in Plate XIV, figure 6. The same dancing granules referred to as occurring in the nuclei of tissue cells prior to the appearance of fully formed polyhedra were also noted within the nuclei of many corpuscles. In diseased caterpillars many corpuscles are encountered which have only one polyhedron, or several, within them (Pl. XIV, figs. 7–9). Such individual or widely separated polyhedra were not formed within the nuclei of the blood cells, but were probably phagocytized by them on escaping into the hæmolymph through rupture of certain tissue nuclei. Another pathological form of corpuscle encountered very often is that shown in Plate XIV, figure 10. Here the entire cytoplasm of the cell seems to have disappeared, having been used, perhaps, as nutriment by the virus, and all that remains is the cellular membrane and a nucleus containing several polyhedra. The writer is certain that this is a pathological condition of the corpuscles, for these cytoplasmic-free elements were never found in normal animals.

As the disease progresses, more and more corpuscles become filled with polyhedra, and the number of polyhedra floating freely in the plasma increases appreciably. As stated above, these bodies escape into the blood through the disintegration of tissue nuclei, so that a large number of polyhedra in the plasma is a good indication of the final stage of the disease within the tissues.

Escherich and Miyajima (4) in their work on *Wipfelkrankheit* consider the blood of nun-moth caterpillars as an absolute and reliable index of conditions existing within the tissue nuclei.   Before using caterpillars for their experiments, these authors repeatedly examined the blood for polyhedra, allowing a stated interval to elapse between successive examinations.   If the last test did not reveal polyhedra within the corpuscles, the caterpillars were diagnosed as healthy; if the blood contained polyhedra, however, the animals were pronounced diseased and were discarded.   Such a procedure consumes a great deal of time, and since time is an important factor in a working season that extends over only eight weeks this method was soon abandoned by the writer in his experiments on gipsy-moth caterpillars.   In order to procure healthy material, a much more serviceable method, described in the section dealing with the infection experiments, was used.   Nevertheless, in order to ascertain the accuracy of the blood test, the hæmolymph of a large series of caterpillars was examined, after which they were sectioned, that conditions within the tissue nuclei might be checked with the appearance in the blood. Table I gives the results of one series of experiments.

TABLE I.—*Results of blood tests and tissue examination of gipsy-moth caterpillars*[a]

Test No.	Examination of blood.	Examination of tissue sections.
1	Moderate number of polyhedra in corpuscles.............	Nuclei filled with polyhedra.
2	.....do..	Do.
3	.....do..	Do.
4	.....do..	Do.
5	Few polyhedra in corpuscles.............................	Do.
6	.....do..	Do.
7	Moderate number of polyhedra in corpuscles............	Do.
8	Large number of polyhedra in corpuscles and many free in plasma.	Do.
9	Few polyhedra in corpuscles.............................	Nuclei normal.
10	.....do..	Do.
11	No polyhedra in corpuscles..............................	Do.
12	.....do..	Do.
13	Moderate number of polyhedra in corpuscles............	Nuclei filled with polyhedra.
14	Large number of polyhedra in corpuscles................	Do.
15	No polyhedra in corpuscles..............................	Nuclei normal.
16	Moderate number of polyhedra in corpuscles............	Nuclei filled with polyhedra.
17	Moderate number of polyhedra in corpuscles and a few out in plasma.	Do.
18	Moderate number of polyhedra in corpuscles............	Do.
19	.....do	Do.
20	Moderate number of polyhedra in corpuscles and a few within the plasma.	Do.
21	Large number of polyhedra in corpuscles and a few out in plasma.	Do.
22	Large number of polyhedra in corpuscles and many out in plasma.	Nuclei filled with polyhedra and many nuclei disintegrating, thus freeing polyhedra.
23	Large number of polyhedra in corpuscles................	Nuclei normal.
24	Large number of polyhedra in corpuscles and many out in plasma.	Nuclei filled with polyhedra and many nuclei disintegrating, thus freeing polyhedra.

[a] The words, "few," "moderate," and "large" in Table I are simply comparative terms that are also used by Escherich and Miyajima (4). A "few polyhedra within the corpuscles" means an occasional find, possibly one or two scattered over many fields examined. By "moderate number" is meant two or three polyhedra within perhaps a third of the corpuscles in a field and represents also a few out in the plasma. Blood classified under "large number" gives a good picture of a caterpillar in the last stages of the disease, for nearly every corpuscle is beset with numerous polyhedra, and, likewise, many are floating about in the plasma.

It will be seen from Table I that the blood, in general, is a fairly reliable index of a caterpillar's condition. Still, it must be noticed that under tests 9, 10, and 23 the conditions in the blood did not represent those of the tissues. However, not a single case was found where polyhedra were in the tissues, but not in the blood. Tests 9, 10, and 23 also indicate that the blood is the first tissue to be affected. This is to be expected, since infection, as will be shown later, occurs by way of the alimentary tract. The virus is ingested with the food and possibly passes through the epithelium of the intestine into the hæmolymph, by means of which it is distributed to the other tissues.

In 1913 both Dr. Chapman and the writer were fully aware of the impracticability of the blood test and also did not consider it an accurate means of diagnosing diseases of caterpillars. After more extensive studies, however, the conclusion has been reached that the blood test, though not irreproachable, is, nevertheless, a better index than it seemed at first. If used at all, the test must be made with extreme care, but, owing to the length of time it requires, it can not be used when one is working with several hundred caterpillars.

ETIOLOGICAL INVESTIGATIONS

In 1913 the problem was again attacked in the most critical manner from the bacteriological point of view. An account of all the work undertaken will not be given, as the results were entirely negative, not essentially differing from those obtained by Jones (10). After a caterpillar has been dead for some time, bacterial invaders enter and cultures are not difficult to obtain; but the writer found, as Jones had, that, when attempts were made to isolate bacteria from caterpillars that had recently died in the field, the tubes remained sterile in most cases, and that, when a growth was obtained, the different species of bacteria isolated behaved as a non-pathogenic intestinal flora. Among all these attempts, no growth was obtained in tubes inoculated with material taken from the body cavity of living diseased animals. The media used, at varying degrees of acidity and alkalinity, included nutrient veal and beef agar and gelatin, bouillon, potato, and caterpillar soup. Anaerobic cultures were also tried, but the results were likewise negative. The bacteriological results bore a striking similarity to those of Tangl (13) and Wachtl and Kornauth (17) with *Wipfelkrankheit* and to the results of Jones (10) in his work on the gipsy moth.

After having satisfactorily eliminated the higher parasites (nematodes, protozoa, etc.) and after obtaining negative results bacteriologically, the following possibilities suggested themselves: (1) Either the polyhedral bodies themselves are parasites; or (2) the etiological factor of the wilt disease is a minute filterable organism independent of the polyhedra; or (3), if the virus is filterable, it may be genetically related to the polyhedra. The filterable-virus viewpoint seemed very promising, especially since

Prowazek (12) concluded from his experiments that it is sometimes possible to infect healthy silkworms with polyhedra-free filtrates. Since Escherich and Miyajima (4) and Glaser and Chapman (6) obtained negative results with Berkefeld filtrates and since Prowazek's filtration results (12) were rather indefinite, the experiments of the writer will be discussed at length, for the reason that their success depended entirely upon proper attention to seemingly insignificant details.

EXPERIMENTATION

Small caterpillars are unfit for experimentation; so it is necessary to wait until they are in the fourth or fifth stage before they can be used. This delay adds somewhat to the difficulty, because at those periods caterpillars are rapidly approaching pupation, and consequently the experimental period becomes shortened appreciably. Furthermore, since the weather is usually hot at this time, wilt is very prevalent in most places, and many more caterpillars are infected than earlier in the season, when the weather is cooler. All the experimental material was obtained directly from the field, as caterpillars raised in the laboratory are unhealthy and utterly worthless.

The first requisite for the experiments was to ascertain whether really healthy material could be obtained. As the lightly infested localities promised a greater number of healthy caterpillars than those heavily infested, a number of lightly infested localities were selected and collections were made, provided the wilt disease was not in evidence. The caterpillars were placed in new wooden boxes and shipped directly to the laboratory. Sometimes the disease broke out during transit; the entire lot was then killed and another collection made. If the insects seemed healthy on arrival, they were placed in autoclaved trays and submitted to a rigid physical examination for four or five days. It has been shown by Escherich and Miyajima (3, 4) and Prowazek (11) that the incubation period of the polyhedral diseases depends very intimately on external conditions; in other words, heat, cold, starvation, poor food, etc., will very soon convert a chronic into an acute case. For this reason these caterpillars were divided into lots and treated in a variety of ways: Some were starved, others were fed with leaves soaked in water for 48 hours, while still others were subjected to the direct heat of the sun. If a single caterpillar died of the wilt disease during the course of any of these treatments, the entire lot was discarded and a fresh collection made; but if all remained healthy, as often proved to be the case, the animals were thought safe to use. This method of obtaining healthy individuals may have its faults, no doubt, but its effectiveness will at once become apparent when the experiments are carefully examined.

Pasteboard boxes, 7 inches long by $5\frac{1}{2}$ inches wide by 5 inches deep, with a cheesecloth lid, were used in all infection experiments. Several

of these boxes can be conveniently placed in an autoclave at one time, and the heat of the steam effectively penetrates through the pasteboard, thus assuring sterilization. After sterilization, a vacuum is produced and the boxes are taken out in just as dry and solid condition as when first put into the autoclave. Every box was autoclaved before using, for, even if new, they may become infected in some way during transit from the factory to the laboratory. After the boxes were removed from the autoclave, they were placed in rooms which had never contained wilt material, and one caterpillar was introduced into each box. In order to have conditions as uniform as possible, all caterpillars belonging to a single experiment were kept in the same room, being fed with red-oak leaves during the entire period. Red-oak leaves remain fresh much longer after being picked than those of either black oak or white oak and were chosen both for this reason and because red oak is one of the most favored food plants of the gipsy moth. To preclude the possibility of introducing the disease by means of the food, the red-oak leaves were shipped daily from a locality not infested by the gipsy moth.

SERIES I

First-stage caterpillars, after having successfully passed their physical examination, were placed in their boxes on June 17 and were infected on June 18. Wilted caterpillars that had been collected a few hours before were ground up in a mortar with just enough sterile water added to facilitate the grinding. The material was then strained through cheesecloth, after which it amounted to 40 c. c., and was divided into two lots of 20 c. c. each. One lot was diluted with 25 times its volume, while the other was diluted with 50 times its volume of sterile water, in order to keep the pores of the Berkefeld filters free. A concentrated, unfiltered emulsion of diseased material is so full of polyhedral bodies, cellular débris, hairs, and pigment granules that it very soon plugs up a filter. Concentrated material, furthermore, is rather thick and slimy, and a film soon becomes deposited on the outside of the candle, thus withholding the virus. Both lots were then thoroughly shaken and filtered through paper filters by means of suction. This filtrate was passed through Berkefeld filters, grade N, and used for the infection experiments.

The filtrates were always controlled culturally and in most cases remained sterile, in so far as bacteria were concerned. One must, of course, be careful to autoclave the Berkefeld filters and all receptacles before using them. One or two cases where bacteria were obtained from tubes inoculated with Berkefeld filtrates could be traced to faulty technique. After shaking the filtrate well, 2 or 3 c. c. were always centrifuged and the bottom sediment examined microscopically for polyhedra; but these were never found, and nothing could be observed, even

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in a dark field, except minute dancing granules, which may be identical with those mentioned under "Pathology of wilt" as having been observed in diseased tissue.

In the first experiment 10 caterpillars were fed with the 1 to 25 dilution passed through a Berkefeld filter, and 10 were fed with the 1 to 50 dilution passed through another Berkefeld filter. Twenty controls accompanied this series.

Ten caterpillars were fed simply with the unfiltered 1 to 25 dilution, while 10 were fed with the 1 to 50 unfiltered dilution. Fifteen controls accompanied this series.

The controls were treated in exactly the same manner as the other individuals, except that they were fed with material that had been sterilized by autoclaving. The infectious material was administered to the caterpillars in two ways: Either some of the liquid was sucked up in a sterile eye dropper, by means of which a drop was placed over the caterpillar's mouth, or the oak leaves were submerged in the material before being placed in the trays. The first method is advantageous, for the reason that a large quantity of the virus can be administered. The caterpillar is held upside down and a drop is placed directly over its mandibles. These and the maxillæ soon begin to move, and gradually the drop disappears. If caterpillars are to be infected in this manner, it is necessary to avoid the period directly preceding a molt, as they will not drink until this process has been completed. Furthermore, if caterpillars have eaten shortly before one wishes to infect them, it is difficult to make them drink; consequently the animals were usually starved for a few hours before infection. The second method is also good, and has the advantage of not consuming as much time as the first. One can be certain whether or not they have eaten by examining the old, infected leaves before replacing them with fresh foliage. In the first experiment both methods were used, and since none died up to June 25, the insects were infected again on that date in exactly the same manner as before.

Fig. 2.—Curve showing the mortality among 20 gipsy-moth caterpillars fed with the wilt virus after filtration through the Berkefeld filter.

In order to determine the effect of temperature on the incubation period,[1] it was recorded daily at noon.

Figure 2 shows the mortality of the 20 caterpillars fed with the Berkefeld filtrate. The ordinate represents the range of temperature between June 18, when the experiment was begun, and July 27, when it was concluded. In order to avoid unnecessary space, the abscissa represents the day when the experiment was begun, the day on which caterpillars died, and the day on which the experiment was concluded.

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[1] The term "incubation period" is used here as the time elapsing between infection and death.

The solid line represents the plot of the deaths from the wilt and the broken line deaths due to another cause. The number of daily deaths is inserted in the squares. When caterpillars died of the wilt disease on a certain day and others succumbed to other causes on the same day, a fraction is used, the numerator representing the number of deaths from the wilt and the denominator the other deaths.

The interpretation of the curve follows: The experiment was begun on June 18; on June 27 one caterpillar died of the "other cause" at 79° F.; on July 1, at 84°, another caterpillar died from the same cause; on July 4, at 83°, one died of the wilt disease and two died of the "other cause;" on July 5, at 85°, two died of the "other cause;" on July 6, at 90°, four died of the wilt; on July 7, at 77°, one died of the wilt; on July 11, at 73°, one died of the "other cause;" on July 14, at 76°, one died of the "other cause;" on July 27, the day the experiment ended, at 80°, one died of the "other cause."

Summing up the results of this experiment, six deaths were caused by wilt, nine deaths were due to the "other cause," one caterpillar escaped during the course of the experiment, and four female moths emerged. Just as soon as a caterpillar died, it was examined for polyhedra, and no death was recorded as having been caused by wilt unless these bodies were in evidence. It is certain that the deaths recorded by the broken line were not due to wilt, because the animals were not flaccid and did not disintegrate on handling; on the contrary, their skin was very elastic and tough, and smears never revealed polyhedral bodies. Many round crystals with radiating lines (Pl. XIV, fig. 11, 12), however, were found. These, the writer believes, are a sign of some metabolic disturbance, and the

Fig. 3.—Curve showing the mortality among the 20 control gipsy-moth caterpillars. Compare with figure 2.

deaths may have been caused by excessive heat coupled with dryness. Laboratory conditions are poor at best, and the food plants in the trays dried out rapidly, although replaced very often by fresh foliage. Thus, it seems that these deaths from another cause are simply a laboratory occurrence, for nothing similar in the field has ever been discovered. The first series of experiments was performed in an excessively hot room below the roof, and, while not a single control insect died of wilt, nearly all the caterpillars succumbed to this general physiological disturbance.

Attempts were made to isolate bacteria from caterpillars which died of wilt in the infection experiments, but in nearly all cases the tubes so inoculated remained sterile. This procedure always gave a good check against the platings made from the infectious material before it was used.

Figure 3 shows the mortality of the 20 controls which accompanied the 20 caterpillars fed with the Berkefeld filtrate. Of these, 19 died on

account of the physiological disturbance and 1 female moth emerged. Figure 4 gives the deaths among the 20 caterpillars fed with the unfiltered material. Of these 17 died of wilt, and 3 of the "other cause." Figure 5 shows the deaths among the 15 control insects that accompanied the caterpillars fed with the unfiltered virus. All 15 died of the "other cause." Table II gives the results of this series of experiments.

TABLE II.—*Mortality among gipsy-moth caterpillars in laboratory experiments (series 1)*

Number of caterpillars.	Treatment.	Died of wilt.	Died of "other cause."	Lived.
20	Berkefeld filtrate..........................	6	9	[a]4 females.
20	Control...................................	None.	19	1 female.
20	Unfiltered virus..........................	17	3	None.
15	Control...................................	None.	15	Do.

[a] Escaped.
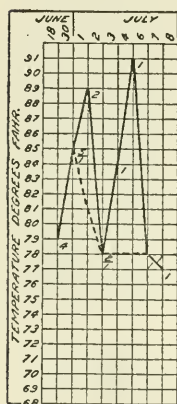
FIG. 4.—Curve showing the mortality among 20 gipsy-moth caterpillars fed with unfiltered wilt virus.

It will be seen that there are almost three times as many deaths from the unfiltered virus as from the Berkefeld filtrate. This shows that the wilt virus is filterable, but with difficulty. Although it would have been much more encouraging to have obtained moths from all the controls, still it is very significant that not a single control insect died of wilt. Noteworthy, also, are the four female moths obtained from the animals fed with the Berkefeld filtrate.

No difference was noted in the rate of deaths between caterpillars fed with the 1 to 25 dilution and those fed with the 1 to 50 dilution.

While the caterpillars in the foregoing experiments were those used in the actual experiments in this laboratory, there were, nevertheless, other caterpillars in trays in the same room. These were held in reserve as stock, but, since not a single one of them died of wilt, they may be regarded also as controls against the experiments, for they were all placed in the trays on the same day the tests were begun (June 18). They were not all the same age, however, and, hence, can not be called controls in the strictest sense of the word.

FIG. 5.—Curve showing the mortality among the 15 control gipsy-moth caterpillars. Compare with figure 4.

Figure 6 gives the mortality among 15 second-stage and third-stage caterpillars. Three were placed in each tray, and all 15 died of the "other cause."

Figure 7 represents the deaths among 26 fourth-stage caterpillars obtained from Providence, R. I., in a lightly infested point which was supposed to be entirely healthy. The disease had not appeared in the field at the time the collection was made, and although all were kept in one tray, not a single caterpillar died of wilt in the laboratory. Twenty-five died of the "other cause" and one male moth emerged.

Figure 8 gives the mortality among 8 fourth-stage caterpillars obtained from Providence. Each caterpillar was placed in a separate tray and the entire eight were starved until they died. The post-mortem appearances were so similar externally to the deaths from the "other cause" that they are represented by a broken line.

Figure 9 represents the deaths among 16 fifth-stage gipsy-moth caterpillars kept in one tray. These were from the same collection as those used in the experiments. Not one case of wilt appeared, but they all died of the "other cause."

Figure 10 shows the deaths among 19 fifth-stage caterpillars, also from the same lot as those used in the experiments.

FIG. 6.—Curve showing the mortality among 15 control second-stage and third-stage gipsy-moth caterpillars.

From figures 2 and 4 it is evident that the deaths from the wilt disease all occurred during days when the temperatures were high. Taking the midway point between 79° and 80° F. in the temperature range 68° to 91°, it is found that 14 deaths occurred at and above 80° against 9 deaths below that temperature. There seems to be no definite correlation between temperature and the deaths from the "other cause."

FIG. 7.—Curve showing the mortality among 26 "control" fourth-stage gipsy-moth caterpillars from Providence, R. I.

SERIES 2

The second series of experiments was begun on July 3. Last-stage caterpillars were used. Material strained through cheesecloth with sterile water to equal 50 c. c. was diluted with 20 times its volume of sterile water, then shaken, and filtered through paper filters. A portion of this filtrate was used as it was, part was filtered through the Berkefeld candles, and another portion was sterilized for the controls. The infections were repeated on July 6, and another dose was administered on July 13 to all caterpillars that had not pupated. These experiments were performed in a room some distance from the one in which the first were carried on.

Figure 11 represents the mortality among 50 caterpillars fed with the Berkefeld filtrate. Wilt caused the deaths of 13 in the caterpillar stage and of 1 in the pupal stage; 3 died of the "other cause";

10 females and 23 males emerged. Figure 12 gives the deaths among 25 caterpillars fed with the unfiltered virus. Twelve died of wilt while caterpillars and four after pupating; three died of the "other cause;" and six male moths emerged. Figure 13 shows the mortality among 25 controls accompanying the series. Three died of wilt, seven died of the "other cause," and twelve males and three females emerged. Table III gives the results of series 2.

TABLE III.—*Mortality among gipsy-moth caterpillars in laboratory experiments*
(series 2)

Number of cater- pillars.	Treatment.	Died of wilt.	Died of "other cause."	Lived.
50	Berkefeld filtrate......................	14	3	{23 males. {10 females.
25	Unfiltered virus......................	16	3	6 males.
25	Control................................	3	7	{12 males. {3 females.
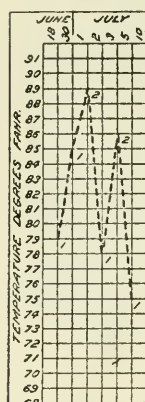
FIG. 8.—Curve showing the mortality among 8 "control" fourth-stage gipsy-moth caterpillars from Providence, R. I.

It will again be noticed that more caterpillars died when fed with the unfiltered material than when fed with the Berkefeld filtrate. If there had been as many caterpillars in the experiments with the unfiltered material as in the Berkefeld-filtrate experiment, the number of deaths from wilt in the former would probably have been more than twice as great as in the latter. Three deaths from wilt are recorded in the controls, but these caterpillars had probably contracted the disease before they were collected, as every possible precaution was taken to avoid contamination. The person who fed the infected individuals was not permitted to feed the controls and whenever a caterpillar died and had to be handled, the hands of the assistant were thoroughly washed in 95 per cent alcohol before being again allowed to handle another tray. In this series of experiments there seems to be no correlation between high temperatures and deaths due to wilt. Taking the middle of the temperature range in the entire series, there are 31 deaths below 79.5° against 2 deaths above 79.5° F. Humidity might prove to be a more important or at least an equally important factor as temperature in determining the progress of the disease; but, since no record was kept of the humidity, the writer is unable to speak with any conviction on this matter.
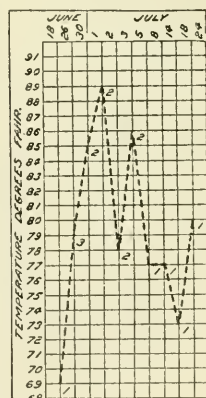
FIG. 9.—Curve showing the mortality among 16 "control" fifth-stage gipsy-moth caterpillars.

SERIES 3

Last-stage caterpillars were used in the third series of experiments, which was begun on July 14. These animals were obtained from a locality in which a tachinid, *Compsilura concinnata*, abounded. Later, during the course of the experiments, quite a large number of these caterpillars succumbed to parasitism by this species.

The wilted material was strained through cheesecloth to equal 50 c. c. This was diluted with ten times its volume of sterile water and filtered through a paper filter. This filtrate was again diluted ten times and a portion was used without further treatment while the rest was passed through Berkefeld candles.

Figure 14 represents the mortality among 40 caterpillars fed with the Berkefeld filtrate. Eight caterpillars died of wilt, seven of the "other cause,"

FIG. 10.—Curve showing the mortality among 19 "control" fifth-stage gipsy-moth caterpillars.

twenty succumbed to tachinid parasitism, and five male moths emerged.

Figure 15 gives the mortality among 40 caterpillars fed with unfiltered material. Fifteen died of the wilt, four died of the "other cause," one escaped, eleven succumbed to tachinid parasitism, and nine male moths emerged.

Figure 16 shows the deaths among 20 control insects. One died of wilt, nine died of the "other cause," one succumbed to tachinid parasitism, and 5 male and 4 female moths emerged. The mortality for series 3 is given in Table IV.

FIG. 11.—Curve showing the mortality among 50 gipsy-moth caterpillars fed with wilt virus after filtration through the Berkefeld filter.

TABLE IV.—*Mortality among gipsy-moth caterpillars in laboratory experiments (series 3)*

Number of cater-pillars.	Treatment.	Died of wilt.	Died of "other cause."	Died of tachinid parasitism.	Lived.
40	Berkefeld filtrate.............	8	7	20	5 males.
40	Unfiltered virus.............	15	4	11	[a] 9 males.
20	Control.....................	1	9	1	5 males. 4 females.

[a] 1 escaped.

Again, the number of caterpillars which died after feeding on the unfiltered material was almost twice as great as the number that died after feeding on the Berkefeld filtrate.

For the sake of uniformity the range of temperature is considered as extending from 71° to 82° F. Naturally the ranges of temperature are not always the same, for one experiment might extend over a greater number of days than another, thus perhaps including a few temperatures different from those in the latter experiment. Taking the midway point in the temperature range as 77°, the number of wilt deaths occurring at and above 77° is found to be 14, while there are 10 occurring below that temperature. There seems to be in this third series of experiments the same correlation between high temperature and wilt as in the first series.
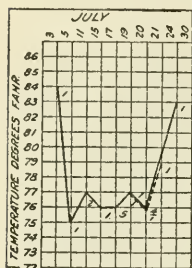
FIG. 12.—Curve showing the mortality among 25 gipsy-moth caterpillars fed with unfiltered wilt virus.

DISCUSSION OF ETIOLOGICAL EXPERIMENTS

In the three series of experiments, out of the entire number of controls, comprising 78 caterpillars, only 4 died of the wilt disease. This is equivalent to about 2.25 per cent, almost a negligible error when compared with about 39 per cent mortality in the infected animals.

It will be seen, on examining the diagrams, that the wilt incubation period varies considerably—from 2 to 27 days. The differences in the individual constitutions of separate caterpillars undoubtedly account for much of this variation, and slight differences in the amount of the infectious material ingested or in the virulence of the virus are important also; but such factors as heat, humidity, and food play a rôle in determining the length of the period of incubation.

FIG. 13.—Curve showing the mortality among 25 control gipsy-moth caterpillars. Compare with figure 12.

FIG. 14.—Curve showing the mortality among 40 gipsy-moth caterpillars fed with wilt virus after filtration through Berkefeld filter.

In spite of apparent infection, a number of moths emerged in each series of experiments. Out of 110 caterpillars fed with the Berkefeld filtrate, 42 adults emerged, while out of 85 individuals fed with the unfiltered material 15 imagoes were obtained. All individuals used in the infection experiments really partook of the material administered, so one can not very well account for the moths on the basis that the caterpillars escaped infection. It is possible that a genetic immunity towards wilt exists among certain members of the gipsy-moth race and that others can also be actively immunized with sublethal doses of fully virulent material.

How can we, however, account for the numerical difference existing between the moths obtained from the Berkefeld-filtrate infections and those obtained from the unfiltered-virus infections? In the experiments with the former, 38 per cent transformed as compared with 18 per cent in the latter case. As the experiments show, the virus is filterable, but with difficulty; consequently naturally unfiltered material would be more deadly than filtered material, for the reason that the former contains a greater number of micro-organisms than the latter, and caterpillars in order to contract wilt would have to ingest more of the Berkefeld filtrate than of the unfiltered virus so as to obtain the lethal dose. This will account for the greater number of moths obtained in the Berkefeld experiments as compared with those obtained in the other infections. In the case of Berkefeld infections the virus was less concentrated and more caterpillars escaped eating the lethal dose, thus perhaps acquiring an immunity toward a second infection, for it must be remembered that most of the individuals were infected a number of times.

Fig. 15.—Curve showing the mortality among 40 gipsy-moth caterpillars fed with unfiltered wilt virus.

Fig. 16.—Curve showing the mortality among 20 control gipsy-moth caterpillars.

All the experimental males and females were mated and eggs were obtained which, it is hoped, will hatch so that they can be used the coming season (1914), in order to determine whether this apparent immunity is racial or merely acquired. If racial, some interesting Mendelian ratios may be obtained; if acquired, the whole of the next generation will probably be susceptible to the disease, unless certain of its members become actively immunized. If racial or acquired immunity does not exist among certain individuals of the gipsy moth, it is difficult to understand how any of these insects escape death under certain conditions in the field. The writer has often seen dozens of caterpillars congregating on trees under burlap and has seen them dying of wilt in great numbers in such places; yet, in spite of the disintegrating bodies flowing out over other individuals in the immediate proximity, many will escape death and transform.

Fig. 17.—Curve showing the mortality among 50 gipsy-moth caterpillars under conditions approximating those in the field.

To repeat this field observation in the laboratory, 50 caterpillars were gathered from a locality where wilt had been raging for several weeks. The entire lot was placed in a single tray so that the dying individuals

could constantly soil the food and infect the living ones. Figure 17 gives the mortality among these insects. Twenty-one caterpillars died of the wilt disease, ten of the "other cause," eleven of tachinid parasitism, and five male and three female moths emerged. The experiment covered a period of 32 days, an ample length of time for every individual in the tray to become infected; yet in spite of the 21 deaths from wilt, 8 moths were obtained.

SUMMARY

(1) The wilt of gipsy-moth caterpillars is a true infectious disease that is distributed over the entire territory infested by the gipsy moth.

(2) Epidemics of the disease occur only in localities heavily infested by the gipsy moth.

(3) Climatic conditions appear to bear an important relation to wilt in the field.

(4) The disease is more prevalent among older than among younger caterpillars, but small caterpillars also die of it in the field.

(5) No diagnosis of wilt is valid unless polyhedra are demonstrated microscopically.

(6) There is no account of the occurrence of wilt in America prior to 1900.

(7) Minute dancing granules may be observed in wet smears.

(8) Polyhedra are probably reaction bodies belonging to the highly differentiated albumins, the nucleoproteids.

(9) The pathology of wilt does not vary with the age of the caterpillars.

(10) The polyhedra originate in the nuclei of the tracheal matrix, hypodermal, fat, and blood cells.

(11) The nuclei of the tracheal matrix and blood cells seem to be the first tissue nuclei affected.

(12) Many minute violently dancing granules are found in the pathological nuclei of fresh tissue.

(13) Giemsa's stain demonstrates many little granules in the nuclei of diseased tissue sections.

(14) The alimentary canal seems to be the last organ in the body to disintegrate.

(15) Two types of blood corpuscles exist in normal hæmolymph.

(16) Two types of pathological blood corpuscles exist in diseased caterpillars.

(17) The blood is a fairly reliable index of a caterpillar's condition.

(18) The blood test is impracticable for large experimental series.

(19) Bacteria are not etiologically related to wilt.

(20) The virus of wilt is filterable with difficulty.

(21) Such a filtrate is free from bacteria and polyhedral bodies.

(22) Caterpillars that have died from infection with filtered virus are flaccid, completely disintegrated, and full of polyhedra.

(23) Minute dancing granules were observed in the Berkefeld filtrate. These may be identical with certain granules observed in smears and tissue nuclei (sub. 7, 12, and 13) and may be etiologically significant.

(24) The incubation period of wilt varies, and temperature at times seems to bear an important relation to this variation.

(25) A large number of caterpillars used in the experiments died of disturbances in their normal physiological activities.

(26) The success of wilt infection experiments is absolutely dependent upon attention to seemingly insignificant details.

(27) Genetic immunity of certain individuals is probable.

(28) Active immunization with sublethal doses is possible.

(29) The polyhedral bodies may be stages of the filterable virus, but as yet no evidence to substantiate this view has been produced.

(30) Infection naturally takes place through the mouth by means of the food.

(31) Some of the imported parasites may be important factors in aiding the dispersion of the wilt disease.

(32) Although probable, there is no definite evidence as yet that wilt is transmitted from one generation to another.

LITERATURE CITED

(1) Bolle, Johann.
  1908. Studien über die Gelbsucht der Seidenraupen. *In* Ber. K. K. Landw.-Chem. Vers. Stat. Görz, 1907, p. 1. *See also* Ztschr. Landw. Versuchsw. Oesterr., Jahrg. 11, Heft 3, p. 279.
(2) Cuénot, L.
  1891. Études sur la sang et les glandes lymphatiques dans la série animale. Pt. 2.—Invertébrés. *In* Arch. Zool. Exp. Gén., s. 2, t. 9, p. 13–90, 365–475, 593–670, pl. 1–4, 15–18, 23.
    Bibliographie, p. 664–670.
(3) Escherich, K.
  1913. Neues über Polyederkrankheiten. *In* Naturw. Ztschr. Forst- u. Landw., Jahrg. 11, Heft 2, p. 86–97, 1 fig.
(4) ——— and Miyajima, M.
  1911. Studien über die Wipfelkrankheit der Nonne. *In* Naturw. Ztschr. Forst- u. Landw., Jahrg. 9, Heft 9, p. 381–402, 6 fig.
    Verzeichniss der zitierten Literatur, p. 401–402.
(5) Forbes, S. A.
  1886. Studies on the contagious diseases of insects.—I. *In* Bul. Ill. State Lab. Nat. Hist., v. 2, p. 257–321, 1 pl.
(6) Glaser, R. W., and Chapman, J. W.
  1912. Studies on the wilt disease, or "flacheria" of the gipsy moth. *In* Science, n. s. v. 36, no. 920, p. 219–224.
(7) ——— ———
  1913. The wilt disease of gipsy moth caterpillars. *In* Jour. Econ. Ent., v. 6, no. 6, p. 479–488.
    Bibliography, p. 487–488.
(8) Graber, V.
  1871. Über die Blutkörperchen der Insekten. *In* Sitzber. K. Akad. Wiss. [Vienna], Math. Naturw. Cl., Bd. 64, Abt. 1, Heft 1/2, p. 9–44, 1 pl.

(9) HOWARD, L. O., and FISKE, W. F.
　　1911. The importation into the United States of the parasites of the gipsy moth and the brown-tail moth. U. S. Dept. Agr. Bur. Ent. Bul. 91, 344 p., 73 fig., 28 pl.

(10) JONES, H. N.
　　1911. Further studies on the nature of the wilt disease of the gipsy moth larvæ. *In* State Forester Mass. 7th Ann. Rpt., 1910, p. 101–105.

(11) PROWAZEK, S. von.
　　1907. Chlamydozoa. II. Gelbsucht der Seidenraupen. *In* Arch. Protistenk., Bd. 10, Heft 2/3, p. 359–364, 2 fig.

(12) ――――
　　1912. Untersuchungen über die Gelbsucht der Seidenraupen. *In* Centbl. Bakt. [etc.], Abt. 1, Orig., Bd. 67, Heft 4, p. 268–284, 1 fig., 2 pl.

(13) TANGL, Franz.
　　1893. Bakteriologischer Beitrag zur Nonnenraupenfrage. *In* Forstw. Centbl., Jahrg. 15, p. 209–230.

(14) TUBEUF, Carl von.
　　1892. Die Krankheiten der Nonne (Liparis monacha). *In* Forstl. Naturw. Ztschr., Jahrg. 1, Heft 1, p. 34–47, fig. 9–10; Heft 2, p. 62–79.

(15) ――――
　　1892. Weitere Beobachtungen über die Krankheiten der Nonne. *In* Forstl. Naturw. Ztschr., Jahrg. 1, Heft 7, p. 277–279.

(16) ――――
　　1911. Zur Geschichte der Nonnenkrankheit. *In* Naturw. Ztschr. Forst- u. Landw., Jahrg. 9, Heft 8, p. 357–377.

(17) WACHTL, F. A., and KORNAUTH, Karl.
　　1893. Beiträge zur Kenntniss der Morphologie, Biologie und Pathologie der Nonne (Psilura monacha L.) ... Mitt. Forstl. Versuchsw. Österr., Heft 16, 38 p., 8 fig., 3 pl.

(18) WAHL, Bruno.
　　1909–1912. Über die Polyederkrankheit der Nonne (Lymantria monacha L.). *In* Centbl. Gesam. Forstw., Jahrg. 35, Heft 4, p. 164–172; Heft 5, p. 212–215; Jahrg. 36, Heft 5, p. 193–212; Heft 8/9, p. 377–397; Jahrg. 37, Heft 6, p. 247–268; Jahrg. 38, Heft 8/9, p. 355–378.

PLATE XI

"Wilted" gipsy-moth caterpillars hanging to a tree trunk.

PLATE XI

PLATE XII

128
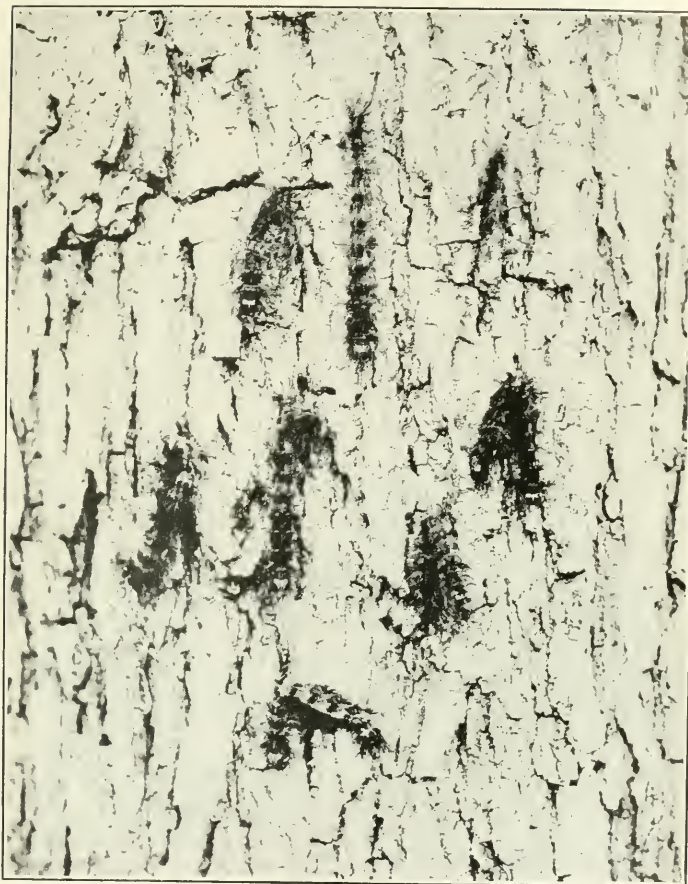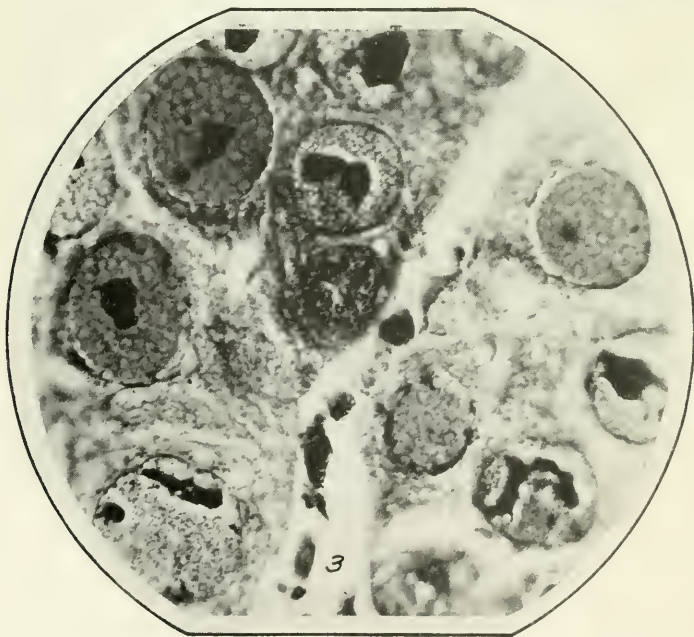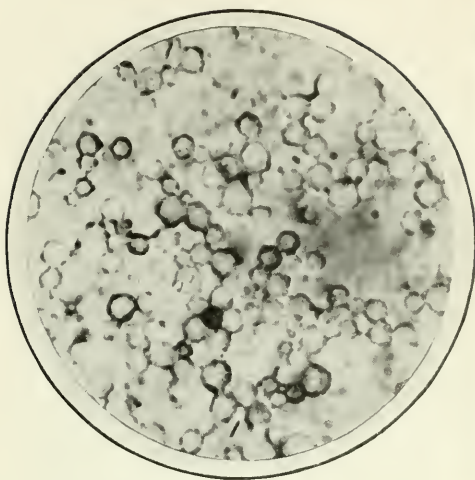
PLATE XII

Fig. 1.—Photomicrograph of a smear from a "wilted" gipsy-moth caterpillar.
Fig. 2.—Photomicrograph of polyhedra clustering around a tracheal tube of a gipsy-moth caterpillar. $\times$ 750.
Fig. 3.—Photomicrograph showing various stages during the formation of polyhedra in tissue nuclei of a gipsy-moth caterpillar. $\times$ 720.

PLATE XIII

Fig. 1.—A silkworm polyhedron, after Prowazek.

Fig. 2.—Two gipsy-moth caterpillar polyhedra adhering to each other.

Fig. 3 to 10.—Polyhedra of gipsy-moth caterpillar cracking to pieces.

Fig. 11 to 18.—Urate crystals of a gipsy-moth caterpillar.

Fig. 19.—Polyhedron of a gipsy-moth caterpillar stained, showing a dark central mass.

Fig. 20.—Polyhedron of a gipsy-moth caterpillar stained, showing refractive granules.

Fig. 21.—Chromatin lump in middle of pathological nucleus of a gipsy-moth caterpillar. × 950.

Fig. 22.—Iron hæmatoxylin showing stained polyhedra of a gipsy-moth caterpillar in a nucleus. × 950.

Fig. 23.—Giemsa's stain, showing unstained polyhedra of a gipsy-moth caterpillar in a nucleus and little granules. × 950.

Fig. 24.—Fully formed polyhedra of a gipsy-moth caterpillar in a nucleus. × 950.

Fig. 25.—Nuclear membrane rupturing and allowing polyhedra of a gipsy-moth caterpillar to escape. × 950.

PLATE XIII
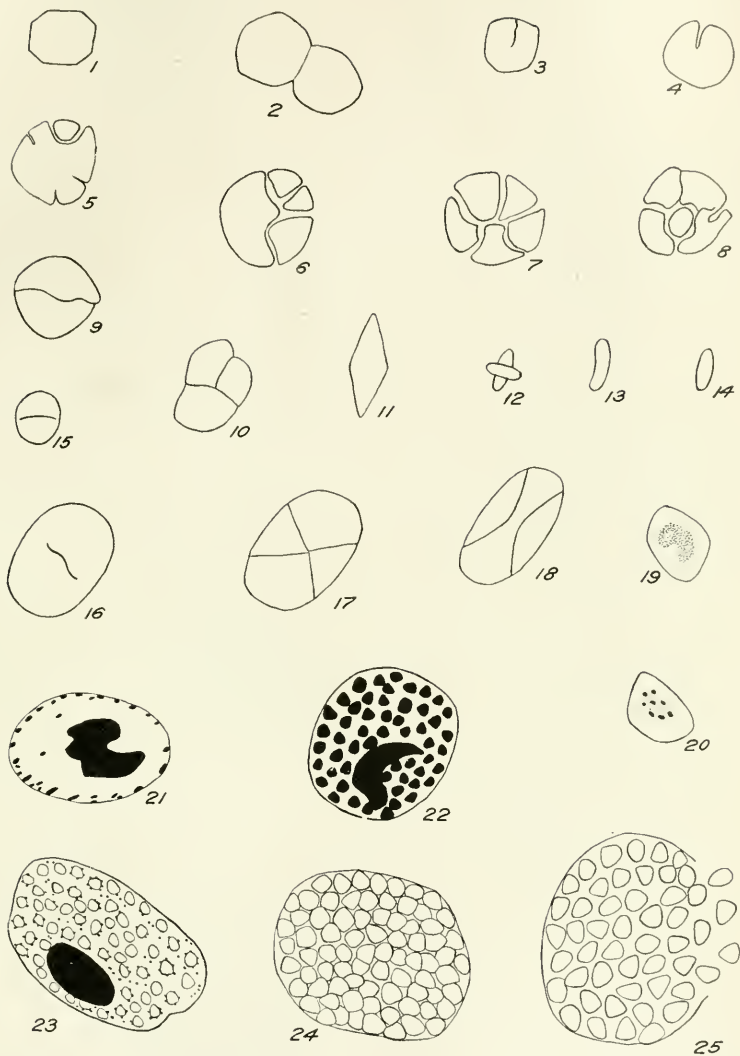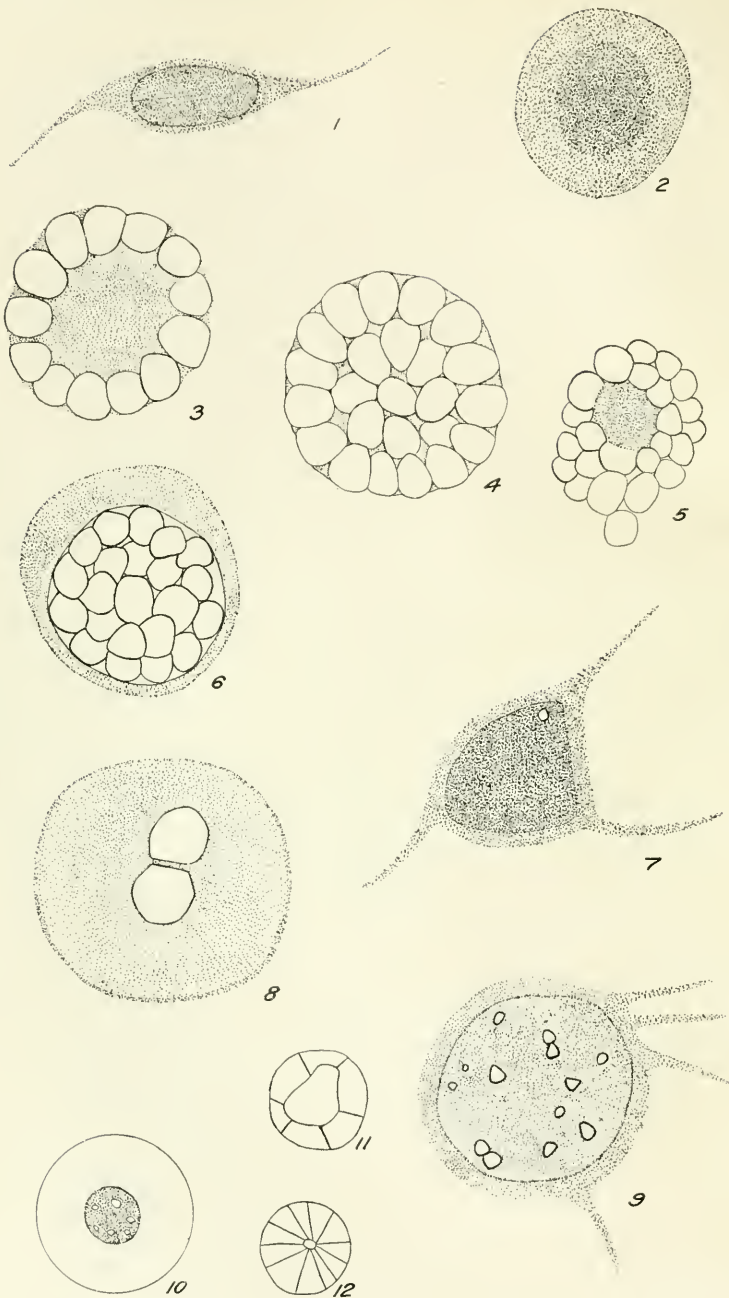
PLATE XIV

PLATE XIV

Fig. 1 and 2.—Normal blood corpuscles of the gipsy-moth caterpillar.

Fig. 3 and 4.—"Mulberry" corpuscles of the gipsy-moth caterpillar.

Fig. 5.—"Mulberry" corpuscle of the gipsy-moth caterpillar crushed, showing nucleus.

Fig. 6.—Blood corpuscle of the gipsy-moth caterpillar, showing nucleus filled with polyhedra.

Fig. 7 to 9.—Blood corpuscles of the gipsy-moth caterpillar with phagocytized polyhedra.

Fig. 10.—Cytoplasmic-free pathological blood corpuscles of the gipsy-moth caterpillar.

Fig. 11 and 12.—Crystals found in gipsy-moth caterpillars that died of the "other cause."

EFFECT OF TEMPERATURE ON GERMINATION AND GROWTH OF THE COMMON POTATO-SCAB ORGANISM

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INTRODUCTION

The causal organism of the common potato scab which has been known to phytopathologists since 1892 as *Oospora scabies* Thaxter was recently pronounced by Lutman and Cunningham[1] as identical with *Actinomyces chromogenus* Gasperini, which was described in 1891. The writer's studies were conducted upon several strongly pathogenic strains isolated from diseased specimens received from Maine, Vermont, and Wisconsin.[2]

All these strains fruit abundantly on the so-called Thaxter's potato agar. The gray film which almost invariably occurs on the scabby spots of naturally or artificially infected tubers when first removed from the soil is made up of the same elements which constitute the fruiting stage in artificial cultures. These elements, called "gonidia," are short, cylindrical segments of aerial filaments and when mature— i. e., when the aerial growth turns from white to dark gray—were employed in making the germination studies here described. They are 1.5 to 2μ long and 0.8 to 1μ broad, with truncate ends. These bodies, after having been sown in agar and shortly before germination, become somewhat broader and rounder, sometimes oval or nearly spherical. Germ tubes may be produced at either or both ends.

EXPERIMENTAL METHODS EMPLOYED

In making the germination tests the ordinary agar hanging-block used in studying the growth of bacteria was employed. A straight transfer needle was rubbed against the surface growth of cultures bearing mature gonidia and then gently drawn across the surface of solidified agar in Petri dishes. The agar blocks for germination studies were then removed from along this inoculated streak and mounted in moist cells

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[1] Lutman, B. F., and Cunningham, G. C. Potato scab. Vt. Agr. Exp. Sta. Bul. 184, 64 p., 7 fig., 12 pl. 1914.

[2] The following method was used to obtain pure cultures from these and numerous other specimens: Both the operator's hands and the diseased tuber are thoroughly washed. Then the latter is rinsed in hydrogen peroxid and dried with sterilized absorbent paper. Next the corky covering of a scabby spot is lifted off by inserting the point of a flamed scalpel under one side of it. The layer of parenchyma underneath is greenish yellow in color, owing to the action of the parasite. The discolored area thus exposed is then gently scraped with a flamed knife and a small quantity (about 1 c. c.) of the pulp transferred to tubes containing 2 or 3 c. c. of sterilized, distilled water. One or more 2 mm. loops of this dilution are transferred to tubes containing 10 c. c. of melted beef agar and the plates poured in the usual way.

in the usual way. Beef-extract agar, without salt,[1] was found to be the most satisfactory medium for the purpose.

Immediately after the hanging-block cultures were made, the slides bearing them were placed in incubators running at the requisite temperatures. They were not removed therefrom except for a short time at the close of each hour for examination with the microscope. To avoid inaccuracies, due to possible variations in temperature in different parts of the incubator chambers, care was taken to see that the temperatures recorded were those in the immediate vicinity of the preparations studied.

THERMAL EFFECT ON GERMINATION

The maximum temperature for growth is apparently a little below 41° C., although occasionally slight evidence of the beginning of germination of gonidia was observed at this point. Germination was most rapid between 35° and 40.5°, and little or no differences were noted in various trials between these limits. At the temperatures mentioned the first evidence of growth was observed at the end of three hours, and below this the time gradually increases: 5 hours are necessary at 30°, 8 hours at 25°, 11 hours at 20°, 18 hours at 15°, and 2 days at 10° C.
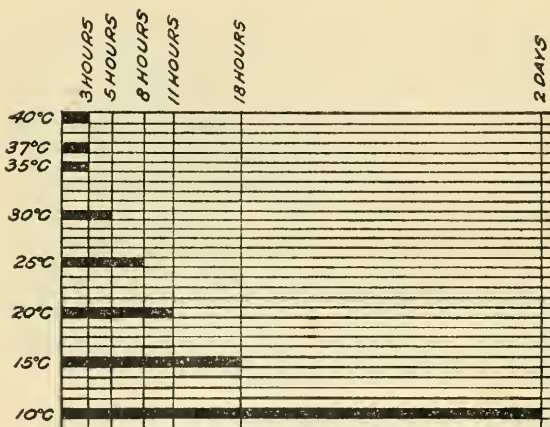
Fig. 1.—Chart showing the relation of temperature to time of germination.

(fig. 1). The largest percentage of germination is usually secured at from 30° to 37° C. Unevenness in germination is evident at 25°, and from this point down it becomes more and more apparent until it is especially pronounced at 10° C. Plate XV, figures 1 and 2, illustrates the characteristic appearance of the germinating gonidia at 35° C.

No attempt was made to determine the exact minimum temperature for germination, but some previous unpublished studies of the writer indicate that it lies somewhere near 5° C. Twenty test-tube cultures, ten in beef broth and ten on potato cylinders, immediately after inoculation with material containing gonidia were placed in a refrigerator where the temperature varied from 5° to 7° C. Only a little growth was noted

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[1] A modification of the formula given by F. D. Chester. (Chester, F. D. Manual of Determinative Bacteriology. p. 28. New York, 1901.)

in a few tubes at the end of one month. A few of the remaining cultures grew when taken to the laboratory, but the rest were dead.

Exposure to cold weather, several degrees below zero centigrade, does not always kill the parasite. During February and March, 1913, many test-tube cultures were exposed immediately after inoculation to freezing at outdoor temperatures and then again taken to the laboratory. The exposure in no case was longer than one week. In no instance were the organisms killed in tubes containing cooked potato cylinders, but in some cases with beef-broth cultures an exposure of five days was fatal when on some nights the thermometer registered as low as −29° C.

RAPIDITY AND VIGOR OF GROWTH

Temperatures between 35° and 40° C. are most conducive to rapid germination. They are decidedly less favorable for the further development of the organism, except that at 35° the growth for the first day was more rapid than at any other temperature tested. No colonies visible to the unaided eye appear in cultures at 39.5°, and growth at this temperature practically ceases within one week. On the other hand, growth is very much retarded and slow below 20° C. Table I shows the comparative rates of germination and growth in cultures at various temperatures and at different intervals within one week.

TABLE I.—*Comparative rates of germination and growth of the common potato-scab organism at various temperatures and at different intervals*

Temperature.	Growth.						
	3 hours.	5 hours.	8 hours.	11 hours.	18 hours.	2 days.	1 week.
° C.							
40	Germination begins.	Threads 2 to 3μ	Very slight progress.	Very slight progress.	Very slight progress.	Threads 5 to 10μ.	Threads 15 to 20μ.
37	....Do....	Threads 5 to 8μ.	Threads 8 to 14μ.	Threads 20 to 22μ.	A network of curled threads.	Small colonies formed.	Feeble colonies of curled threads.
35	....Do....	Threads 5 to 14μ.	Threads 15 to 20μ.	Threads up to 70μ.	A complete network.	Colonies formed.	More or less complete growth along the line of inoculation.
30	....	Germination begins.	Threads 5 to 8μ.	Threads 25 to 30μ.	A network..	.....do......	Do.
25	....	....	Germination begins.	Threads 11 to 22μ.	.....do......	.....do......	Do.
20	....	....	....	Germination begins.	Threads up to 50μ.	Formation of colonies.	Do.
15	....	....	....	....	Germination begins.	A network..	Formation of colonies.
10	....	....	....	....	....	Germination begins.	Threads 27 to 30μ.

Observations upon cultures for longer periods, two to four weeks, indicated that the optimum temperatures for maximum growth are from 25° to 30°, with practically no difference between. The total growth produced was less above and below these points. While it was still proceeding normally but at a slower rate at the lower temperatures, at 35° it was not only less but appeared to have reached its end.

The discoloration of the medium which is very characteristic of cultures of this parasite was faint or absent at and above 35° and quite intense at and below 30° C.

INVOLUTION FORMS

High temperature is considered one of the factors influencing the production of degeneration forms of bacteria,[1] but this is apparently not the case with the potato-scab organism. While the individual filaments, which at lower temperatures are long and more or less curved, appear very short and curled at 37° C., and especially so at 39° and 40°, the writer does not consider them strictly involution forms. The gonidia, also, can not be considered as involution forms, since morphologically the same bodies occur normally upon scab spots on potato tubers and apparently serve as fruiting organs.

However, such abnormal growths may be produced by certain kinds of culture media. The writer has observed some very interesting involution forms which constantly appear at all temperatures when the scab organism is grown upon a synthetic agar that is much used in this laboratory for the cultivation of fungi.[2]

On this medium germination and growth proceeds normally at first, but after two days, if incubated at 35° to 37°, which, as has already been pointed out, are within the range of most favorable temperatures for germination and early growth, the threads become distorted and swollen at various places, both at the tips and in the middle. Sometimes even the gonidia themselves become abnormally enlarged at or before germination. At the end of a month the entire growth will consist of swollen, club-shaped, oval, or spherical segments of various sizes (Pl. XV, fig. 3 and 4). Not infrequently these abnormalities reach 4μ in diameter. The consistency of the growth thus produced is soft and somewhat slimy instead of being tough and hard, as is usually the case. By leaving out one of the ingredients of the medium at a time it was

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[1] Migula, Walter. System der Bakterien . . . Bd. 1, p. 52–53. Jena, 1897.

[2] This synthetic agar was prepared according to the formula given by Darwin and Acton (Darwin, Francis, and Acton, E. H. Practical Physiology of Plants. ed. 3, p. 68. Cambridge, 1909) and consists of—

	Gm.		Gm.
Dextrose	50	Magnesium sulphate	2. 5
Peptone	20	Potassium monophosphate	2. 5
Ammonium nitrate	10	Calcium chlorid	0. 1
Potassium nitrate	5	Distilled water	1,000

found that no such involution forms were produced when the potassium monophosphate alone was excluded, but they invariably appeared if it was present.[1]

SUMMARY

(1) Temperatures from 35° to 40° C. are most favorable for the germination of the gonidia of the potato-scab organism. They are unfavorable for long-continued growth, although at 35° a stimulating effect was produced at first.

(2) The maximum temperature for growth is about 40.5°, the optimum 25° to 30°, and the minimum about 5° C.

(3) Involution forms are produced, but not as the result of temperature conditions. They appeared abundantly when 0.25 per cent of potassium monophosphate was included in a synthetic culture medium.

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[1] Münter observed similar involution forms in pure cultures of certain soil-inhabiting Actinomyces, but on a somewhat different synthetic medium than that used by the writer. Among the species studied by Münter *Actinomyces chromogenes* is mentioned. (Münter, F. Über Actinomyceten des Bodens. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 36, No. 15/18, p. 380-381. 1913.)

PLATE XV

Fig. 1.—Germinating gonidia of the potato-scab organism, agar hanging-block, 3 hours' incubation at 35° C. ×375.

Fig. 2.—Germinating gonidia of the potato-scab organism, agar hanging-block, 5 hours' incubation at 35° C. ×375.

Fig. 3.—Involution forms of the potato-scab organism on synthetic agar from a 1-month-old culture, stained with carbol fuchsin. ×425.

Fig. 4.—Involution forms of the potato-scab organism on synthetic agar from a 1-month-old culture, stained with carbol fuchsin. ×750.

SEEDLING DISEASES OF SUGAR BEETS AND THEIR RELATION TO ROOT-ROT AND CROWN-ROT [1]

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INTRODUCTION

The diseases of the sugar beet (*Beta vulgaris L.*) to be discussed in this paper are damping-off and a more common but less familiar seedling trouble which will be designated as "root sickness," together with the associated rots of the growing or of the mature root.

Damping-off is a disease which typically manifests itself by a characteristic browning or blackening of that portion of the root or hypocotyl near the surface of the ground. Plants may either be killed by the progress of the disease or checked in growth for a longer or shorter time, according to the severity of the attack and the environmental conditions. The trouble is found in incipient form wherever the host is cultivated and not infrequently becomes epidemic, completely destroying the stand.

Root sickness is similar in some respects to damping-off, but the attack in this case is confined to the root system, seldom appearing aboveground. For this reason it has heretofore escaped general recognition as a distinct disease and has received no treatment in American literature. Diseased plants assume a slightly flabby appearance and are perhaps a trifle lighter green than normal ones. In severe cases the entire field may be wiped out, but more frequently enough plants survive to produce at least a partial stand. The beets make practically no growth during the continuance of the attack, which is usually for two or three weeks. By carefully removing the sick plants from the soil so as to avoid breaking the roots it may be seen that the side branches and taproots are blackened, shriveled, and more or less completely killed. Healthy new shoots are sent out here and there from the upper portion in an attempt at recovery. If the plant survives, one of these eventually replaces the taproot. However, such beets are not only delayed two or three weeks in growth but they are likely to be short and branching. The stand is always more or less imperfect in fields that have recovered from the disease. This type of trouble is almost universal, and in some of the heavier soils of the more northern areas it frequently becomes a limiting factor in sugar-beet production. Even in the more favored sections it is annually the cause of

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[1] The major portion of the work reported in this paper was carried out at Madison, Wis., in cooperation with the Wisconsin Agricultural Experiment Station, which kindly supplied space and care for the field plots and provided all necessary laboratory and greenhouse facilities.

serious losses. Many of the failures attributed to faulty germination are, in fact, the results of serious outbreaks of this disease, and in practically all the cases investigated where seedlings were "not growing well" the trouble has been found to be root sickness.

These or similar diseases have been known for a long time in Europe, where various and widely different theories have been advanced regarding their cause or causes. Refractory soil, cold ground, wet weather, poor cultivation, excessive rain, fungus infection, and the like have all had their advocates, who have based their opinions in many instances upon insufficient data.

Hesse (24) [1] reported the presence of *Pythium debaryanum* in diseased beet seedlings in 1874, but he seems to have done no experimental work with this host. Hellriegel (23), one of the first investigators who made a careful experimental study of this subject, showed that damping-off in his pot experiments appeared to be due to a parasite, and traced the source of infection to the seed. He did not, however, assign a specific organism as the cause. Eidam (13) produced artificial infection of beet seedlings with cultures of *Rhizoctonia betae* Kühn. Krüger (25, 26) found *Phoma betae* Fr. to be an active seedling parasite of the sugar beet and expressed the opinion that several different fungi are capable of producing diseases in seedlings of this plant so similar in appearance that they have been classed together under the name "Wurzelbrand." Several fungi, as well as bacteria, have since been added to this list of parasites, while other writers have denied the parasitic origin of the disease.

So much contradiction and uncertainty exists in the literature of the last 20 years regarding the nature and cause of Wurzelbrand of beets in Europe that Peters (31, 32, 33) and his associates (5, 6) found it necessary to go over the entire subject and submit to rigid experimental proofs the more worthy of the hypotheses that have been put forth.

From what appears to be a careful and trustworthy piece of work, Peters (33) concludes that under the conditions of his experiments *Pythium debaryanum* Hesse, *Phoma betae* Fr., and *Aphanomyces laevis* De By. are capable of producing damping-off of the sugar beet. He was unable to secure pure cultures of *Rhizoctonia violacea* Tul., but he infected soil with fragments of beets showing typical Rhizoctonia decay and failed to produce damping-off. The evidence of the parasitism of bacteria on this host seemed to him insufficient to justify serious consideration.

In America the only reported work on seedling diseases of beets, except the author's preliminary note (12), is by Duggar (9, 11), who conducted successful infection experiments with a species of Rhizoctonia that he secured from decayed beets and later designated as *Corticium vagum* B. and C., var. *solani* Burt. (10, p. 444–452). His experiments were carried out in sterilized soil and controlled by check plants

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[1] Reference is made by number to "Literature cited," p. 165–168.

which remained healthy, but he makes no mention of treating the seed to insure the elimination of *Phoma betae*, which he regarded as absent (10, p. 344).

THE SECURING OF CULTURES

The first steps in the present work were naturally the securing of cultures, which were obtained in various ways. Beet seed was placed in sterilized filter papers in sterile moist chambers, and transfers were made from the colonies of fungi which developed during the progress of germination. This method is uncertain and yields a large number of harmless saprophytes. Seed was sown in soil which had been sterilized in an autoclave at 12 pounds' pressure for from four to six hours on two successive days. These were watered with sterile water and protected from outside sources of infection. Whenever damping-off appeared, the diseased seedlings were removed and treated for one minute with more or less shaking in a solution (1 : 1,000) of bichlorid of mercury in water or in similar bichlorid solutions containing either 1 gm. of ammonium chlorid or ½ c. c. of concentrated hydrochloric acid per liter. They were then rinsed in sterilized water and dropped upon suitable nutrient-agar medium in Petri dishes (Pl. XVI and XVII). The agar most commonly used is sufficiently acid to materially check the development of bacteria and is at the same time a very satisfactory medium for the cultivation of most fungi. It has the following composition:

Dextrose	100	Dipotassium hydrogen	
Peptone	5	phosphate	2. 5
Ammonium nitrate	10	Calcium chlorid. :	. 1
Potassium nitrate	5	Water	1, 000
Magnesium sulphate	2. 5	Agar	20

As soon as growth appeared from the seedlings, isolation transfers were made. Cultures obtained by these two methods were regarded as originating from the seed.

Cultures were made from the soil indirectly by means of seedlings in the following manner: Beet seed, treated by a method to be discussed later, so as to insure the absence of parasitic fungi, was sown in unsterilized soil in pots that were thoroughly sterilized before using. When damping-off occurred, isolations were made in the manner already described. Cultures were secured from decayed beets by cutting out with a sterile knife small portions of material on the border line between healthy and diseased tissue. These blocks were placed upon a suitable medium, and isolations were made from the developing colonies. Another method employed was to sow treated beet seed in sterilized soil, subsequently infected with fragments of decaying beets. Isolations were then made from the seedlings when disease developed. Large numbers of isolations were made from sugar-beet seedlings grown in commercial fields, and a considerable number of cultures were courteously contributed by various workers from time to time.

SEED TREATMENT

Sugar-beet seed is quite universally infected with parasitic fungi. It was therefore necessary to devise some method of freeing the seed from infection before inoculation experiments could be successfully conducted. Among the substances tried were hydrogen peroxid, hydrochloric acid, sulphuric acid, formalin solution, formaldehyde vapor, and hot water. Peroxid solution (6 per cent) was used for varying periods up to one hour. The seed was then sown in sterilized soil and watered with distilled water. The pots were protected from infection, but damping-off was in no degree checked, and *Phoma betae* was invariably isolated from the diseased seedlings (Pl. XVII).

Hydrochloric acid was employed in various concentrations up to a specific gravity of 20° B. for 15 minutes. The seed was then rinsed in sterile water, followed by lime water, which in turn was followed by sterile water. This treatment was without effect upon the vitality either of the seed or of the fungi.

Sulphuric acid was used in various strengths up to full concentration for one hour. The treated seed germinated strongly and from 24 to 48 hours earlier than the untreated control, but there was no decrease in the amount of damping-off.

Formalin solution was employed up to concentrations of 2 per cent of formaldehyde for various intervals up to one hour. This seriously injured the viability of the seed, but afforded no check to the disease. The results with formaldehyde vapor are inconclusive, since they lack uniformity.

The method finally settled upon for experimental work was one employed by Peters (7, p. 273–274), which consists of heating the seed in water at 60° C. for 10 minutes, promptly drying superficially upon filter papers, so as to prevent germination, and after an interval of 24 hours heating a second time for 10 minutes at 60° C. The seed treated in this way and sown in sterilized soil, watered with sterile water, and protected from outside infection remained practically free from disease. Not more than one seedling in three or four hundred was infected with *Phoma betae*. The percentage of germination is unquestionably lowered by this treatment, but it is the only method tried by which inoculation experiments could be controlled. It does not appear to be a method which could be applied on a commercial scale.

METHODS OF INOCULATION

Inoculation experiments were carried out in pots either in the laboratory or usually in the greenhouse, using seed treated in the manner just described, and with soil sterilized by heating three or four hours in the autoclave under a pressure of from 12 to 15 pounds on two, or usually three, consecutive days. Inoculations were made in the soil or upon the seed at the time of sowing, except when otherwise stated. Various

methods of inoculation were employed, but the results were uniformly the same. Either suspensions of spores or mycelial growth on various culture media, such as agar, corn meal, beet petioles, and sterilized beet blocks, were employed. The last method—that is, with beet blocks—was perhaps the most satisfactory and convenient. The corn-meal cultures appeared to exercise an unfavorable physiological action, possibly because of the bacterial growth which they fostered; so this method was discarded.

Inoculation experiments were invariably controlled by a considerable number of uninoculated pots. In a few instances disease occurred in the controls. In such cases the causal organism was determined, but the entire series was abandoned as an inoculation experiment, even though the presence of an intruder could be explained readily through the agency of insects and earthworms. It was the invariable custom to recover the fungus from the damping-off seedlings by the method already described (Pl. XVI and XVII) and to reinoculate and recover through from four to six generations of seedlings.

As reported in a former note (12), four fungi have been found to stand in causal relation to seedling-beet troubles. These are *Phoma betae* (Oud.) Fr., a species of Rhizoctonia, regarded as identical with the form described as *Corticium vagum* B. and C., var. *solani* Burt., *Pythium debaryanum* Hesse, and a fungus originally reported as *Aphanomyces laevis* De By., but which has since been found to be new.

PHOMA BETAE

TAXONOMY

Frank (16, 17, 18, 20) established the relation of *Phoma betae* to heart-rot of the sugar beet in 1892. The following year Krüger (25, 26), working in the same field, demonstrated its causal relation to damping-off. He found the fungus fruiting abundantly on all parts of diseased beets and held it to be identical with the fungus which had previously been observed on various portions of the cultivated varieties of *Beta vulgaris* L.

Oudemans (29, p. 181) had observed what appeared to be the same fungus fruiting upon leaf spots of old beets and applied the name "*Phyllosticta betae*." Prillieux (35, p. 19) observed the fungus on leaf blades and decaying heart leaves, as well as upon typical spots on the leaf, and applied the name "*Phyllosticta tabifica*." Saccardo recognizes the names "*Phyllosticta betae* Oud." and "*Phoma betae* Rostr." The latter name is given on the authority of the following paragraph from the pen of E. Rostrup (41, p. 323):

Eine zweite, an Runkelrüben auftretende *Phoma* habe ich zuerst in meinem Jahresbericht über Krankheiten der Kulturgewächse im Jahre 1888 (Tidsskrift for Landökonomi. R. 5, Bd. 8, S. 746) [40] unter dem Namen *Phoma sphaerosperma* beschrieben. Weil sich aber herausstellte, dass dieser Name schon im Jahre 1885 einer ganz anderen Art gegeben war, nannte ich später den Pilz *Phoma Betae*.

No reference to the publication in which this change of name was announced is given, and efforts to find it have failed. Moreover, Lind (28, p. 415), who had full access to Rostrup's specimens and publications, lists *Phoma betae* Rostr. under *Phoma betae* Fr. as a synonym. Frank suggested the name "*Phoma betae*" in the article previously cited (16), published in 1892, which contains a description of the fungus, accompanied by figures and a somewhat extended discussion of the root-rot produced by it on the sugar beet. In view of the established identity between Phoma and Phyllosticta on sugar beets it would appear that Oudemans' description in 1877 (29) has first claim to priority.

For these reasons it seems proper and convenient to retain the name which has persisted in most general use in literature, but with a correction to insure proper acknowledgment to Oudemans. The name "*Phoma betae* (Oud.) Fr.*" is therefore used in this paper. Pool and McKay, who agree in the justice of this usage, also employ it in a current paper (34).

Neither Frank (17) nor Krüger (25, 26) were able to find evidences of a perfect stage of this fungus in their cultural studies. Peters (31, 32, 33) also failed to find sexual fruits, and the same is true of the work of the writer. It should be pointed out, however, that Rostrup (40, p. 746) believed *Sporodesmium putrefaciens* Fuck. to be a perfect stage of *Phoma betae*, and Prillieux and Delacroix (37) regarded *Phoma betae* as the pycnidial form of *Sphaerella tabifica* Delacr.

IDENTITY OF PHOMA AND PHYLLOSTICTA ON THE SUGAR BEET

Hedgcock (22) has presented evidence by cross-inoculation of the identity of Phyllosticta and Phoma on the sugar beet. He grew beets from seed treated with concentrated sulphuric acid for 30 minutes, followed by an alkali, and successfully produced Phyllosticta spots by spraying upon the leaves spores of a Phoma culture isolated from decaying beets. Beets whose leaves were covered with Phyllosticta were placed in a dry cellar and held under observation for two months, during which time the characteristic black rot of Phoma passed from the leaf petioles to the crown of the beet.

While Hedgcock's conclusions are undoubtedly correct, the seed treatment he employed does not destroy the viability of Phoma, and it will be shown later that beets whose foliage was free from visible evidence of Phyllosticta decayed from Phoma when they were placed in a relatively dry environment. The case may be strengthened, therefore, by the presentation of the additional evidence now available. Cultures from Phyllosticta spots, as well as from decayed beets and from damped-off seedlings, have been found equally capable of producing damping-off of sugar beets, and a somewhat extended study of the morphology of the fungus from the three sources mentioned has revealed no differences

between them. The Phyllosticta cultures employed were supplied by Miss Venus W. Pool, of the Rocky Ford (Colo.) field station. Pool and McKay (34), who worked with Phoma cultures isolated by the writer from decayed sugar beets, found them capable of producing the characteristic leaf spots as readily as cultures isolated from the Phyllosticta pycnidia. The fungus, however, is not an aggressive leaf parasite, but does its greatest injury on the root.

SOURCES OF INFECTION

The source of original infection appears to be the seed. It has been generally recognized in Europe for years that seed infection with *Phoma betae* is universal. As American growers are using European seed almost exclusively, it follows that the disease is constantly being introduced into the United States on seed. A very large number of samples of both European- and American-grown beet seed have been examined for the presence of this and other pathogenic forms. With the exception of one single lot of seed, the examination of 100 seed balls by the seedling method has invariably demonstrated the presence of *Phoma betae*. Reexamination of this one lot, which was American-grown, revealed the presence of the fungus in it also when larger samples were tested.

Frank (18, p. 180, 272–293) believed the fungus capable of living over in the soil by means of its spores, but Peters (7, p. 278–286) holds that it can do so only when fragments of beets are present to support mycelial growth. A large number of trials of soil in America made by means of seedlings growing in it from pasteurized seed indicate that Phoma does not remain viable in the soil after the decomposition and disintegration of its host. Field soils containing decaying beet fragments occasionally yield cultures of the fungus in the spring of the first year following beets, but, as a rule, even seriously beet-sick soils fail to give them. Samples have been examined from Virginia, District of Columbia, Michigan, Wisconsin, Kansas, Colorado, Utah, and California.

MORPHOLOGY OF THE FUNGUS

Several hundred cultures of the fungus have been isolated and grown upon media (Pl. XVII). No constant differences in cultural characters of strains from the various sources have been observed. It is readily cultivated upon a great variety of media, although on many of these it develops mycelium only. It fruits abundantly upon string-bean agar (Pl. XVII, fig. 2) and this medium has been used for purposes of identification and for measurements of pycnidia and spores. It is evident that the curves which might be plotted from the following tabulated results of measurements would be irregular and consequently that they would probably be changed by increasing the number of pycnidia and spores

measured (Table I). Of 181 pycnidia measured the smallest was 125μ and the largest 635μ in diameter. The largest number fall between 225 and 325μ.

TABLE I.—*Variation in size of 181 pycnidia of Phoma betae*

Number measured.	Variation in diameter.	Number measured.	Variation in diameter.
	μ		μ
1	125	11	351 to 375
4	126 to 150	5	376 to 400
14	151 to 175	10	401 to 425
7	176 to 200	5	426 to 450
31	201 to 225	1	451 to 475
21	226 to 250	1	476 to 500
10	251 to 275	1	501 to 525
27	276 to 300	1	526 to 550
19	301 to 325	1	551 to 635
11	326 to 350		

The pycnospores showed quite wide variations in size. The shortest of 204 spores measured 3.8 and the longest 9.4μ. Practically all fell between 4.1 and 7μ, as shown in Table I.

The width of the pycnospores varied from 2.6 to 4.3μ. The single exception to this case is a spore which measured 4.9μ in width. Table II will show the distribution of numbers within the limits given.

TABLE II.—*Variation in size of 204 pycnospores of Phoma betae*

LENGTH

Number measured.	Variation.	Number measured.	Variation.
	μ		μ
1	3.8	14	6.1 to 6.2
4	4.1 to 4.2	11	6.3 to 6.4
3	4.3 to 4.4	6	6.5 to 6.6
2	4.5 to 4.6	6	6.7 to 6.8
14	4.7 to 4.8	7	6.9 to 7.0
13	4.9 to 5.0	1	7.1 to 7.2
22	5.1 to 5.2	1	7.3 to 7.4
21	5.3 to 5.4	2	7.5 to 7.6
24	5.5 to 5.6	2	7.7 to 7.8
22	5.7 to 5.8	1	7.9 to 8.0
25	5.9 to 6.0	2	9.3 to 9.4

WIDTH

Number measured.	Variation.	Number measured.	Variation.
5	2.6 to 2.7	22	3.6 to 3.7
15	2.8 to 2.9	17	3.8 to 3.9
43	3.0 to 3.1	7	4.0 to 4.1
49	3.2 to 3.3	3	4.2 to 4.3
42	3.4 to 3.5	1	4.9

VITALITY IN CULTURE

Phoma betae exhibits long vitality in culture, as is shown by the following tests.

On July 2, 1913, old cultures which had been apparently air-dry for months were opened under sterile conditions, and into each a portion of string-bean agar was introduced. The tubes were then placed to harden in such a position as to leave the old culture partly submerged. Table III gives the age of the cultures and the results after six days' incubation.

TABLE III.—*Vitality of Phoma betae in culture*

Phoma strain.	Number.	Age of culture.	Results.	Phoma strain.	Number.	Age of culture.	Results.
		Days.				*Days.*	
Strain 3...	39	413	Good growth and pycnidia.	Strain M..	1,304	315	Good growth and pycnidia.
Strain A...	423	372	Do.	Strain 3...	64	413	No growth.
Strain J...	564	358	Do.	Strain 3...	40	413	Do.
Strain J..	996	315	Do.	Strain 3 ..	281	404	Do.
Strain E..	1,296	315	Do.	Strain 3 ..	386	404	Do.
Strain J..	1,301	315	Do.				

INOCULATION EXPERIMENTS ON SEEDLINGS

The strains used in inoculation experiments are 24 in number, obtained from the following sources:

Direct isolation from sugar-beet seed by the moist-chamber method, 1.

Indirect isolation from sugar-beet seed through damping-off seedlings in sterilized soil, 2.

Direct isolation from Phyllosticta leaf-spot, 2.

Isolations from various sources such as beet leaves, beet seed, and beet-sick soil at Rocky Ford, Colo., 13.[1]

Isolations from beets having heart-rot, from Colorado, 1; Wisconsin, 2; South Dakota, 1.

Isolations from seedlings grown in sterilized soil, inoculated with decayed beets, 2.

The inoculation experiments upon seedlings were carried out in the manner already described, invariably yielding positive results. There appeared to be no decrease in virulence from carrying the fungus in culture 14 months.

The first development of damping-off in the pots occurred usually about the third day after the seedlings broke the ground and continued till about the time they developed their third or fourth pair of leaves (Pl. XVIII, fig. 2). The method followed was to sow 100 seed balls per

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[1] These cultures were kindly supplied by Miss Venus W. Pool, of the Bureau of Plant Industry, from the Rocky Ford (Colo.) field station.

pot and to examine daily for damping-off. Whenever disease occurred, the plants affected were removed and a record made of their number. The period of greatest susceptibility seemed to be passed by the time the third set of leaves appeared. After a sufficient period the remaining plants were harvested and examined for signs of infection on the roots.

The following record (Table IV) of a typical series illustrates the method and gives a good idea of the average results.

TABLE IV.—*Results of inoculation experiment with Phoma betae*

[Series of June 27, 1912]

Phoma strain.	Appeared above-ground on—	Number of seedlings diseased on July—									Number harvested on July 29.	
		3	4	5	6	7	10	12	15	17	Diseased.	Healthy.
Strain C	July 1	....	....	3	5	6	18	19	5	0	4	18
Strain E	...do....	....	7	10	7	8	9	7	3	2	5	3
Strain B	...do....	....	2	3	6	3	12	13	4	4	7	30
Strain A	July 2	....	1	2	8	12	27	17	6	2	7	13
Strain D	July 1	2	24	20	13	8	16	2	0	0	1	0
Strain F	...do....	....	....	....	5	6	9	9	10	1	5	14
Strain H	...do....	....	7	44	12	0	11	0	0	2	.....	.....
Strain G	...do....	....	....	....	8	0	21	18	14	0	52	12
Do	...do....	....	5	11	22	45	5	0	0	0	5	7
Control	July 2	....	....	....	....	....	....	....	....	....	.....	111
Do	...do....	....	....	....	....	....	....	....	....	....	.....	148

The plants described as diseased were typical root-sick beets. There was nothing about their appearance aboveground in the pots to indicate the presence of the fungus, but in the field such seedlings may often be detected from the fact that they appear to be suffering from lack of moisture. This type of disease is almost universal under field conditions and is far more common than damping-off. Histological studies upon such material, to be published in a subsequent paper, revealed the significant fact that it often carries the pycnidia and sometimes the vegetative mycelium of the fungus.

The number of seedlings developing in control pots was, as a rule, larger than the total number developing in inoculated ones. This is to be explained by the fact that some of the seedlings were attacked before they broke the ground and in consequence were unable to push through the soil. A careful examination of the surface soil in inoculated pots now and then revealed such seedlings in a state of more or less complete decay.

PHOMA FIELD-ROT AND STORAGE-ROT FOLLOWING SEEDLING INFECTION

When diseased plants were allowed to remain in the pots and were held under as favorable conditions as possible, a large proportion of them eventually survived and sent out new roots. This condition is

often observed in commercial fields, and it is probably safe to say that, under advantageous conditions of climate and cultivation, 75 per cent of the beets attacked by *Phoma betae* are not killed, but throw off the parasite sufficiently to make a good growth while continuing to carry the fungus in a dormant condition upon the crown. When the vitality of the host is sufficiently reduced, the parasite may become active again and develop either in the root, where it causes a black rot, or on the aerial portions of the plant, where it causes a spot disease, which, while it does not injure the host to an appreciable extent, enables the fungus to infect the seed growing on mother plants. The rot is, of course, more serious to the plants attacked. Cases of this so-called heart-rot in the field have been very destructive at times in certain sections of Europe and are not infrequent in America. Important instances of its occurrence in Michigan, Wisconsin, and Colorado, and a less serious case in New York, have come to the writer's attention. Material from the three States first mentioned was available for study. The fungus was easily secured in pure culture from the beets that were partially decayed by the black rot typical of the disease (Pl. XVIII, fig. 1). Many roots presented unmistakable evidence of having been diseased in the seedling stage. In some cases the original taproot had been destroyed, and only a knob of tissue just below the crown had survived.

Additional evidence that the fungus is capable of living upon its host for a long time in an inconspicuous and apparently harmless form until those conditions appear which are favorable to its development is readily found upon examination of beets in storage or those which have been kept over for seed purposes. Such examination in the spring has never failed to reveal the presence of the fungus. Sometimes the entire root is destroyed, but more frequently beets that are apparently healthy may be found to show black spots in certain sections in or near their vascular tissue, especially in the top of the crown. These areas usually develop longitudinally and are frequently confined to the vicinity of a single vascular bundle (Pl. XIX). When the conditions of storage have been unfavorable, the rot may assume an epidemic form and lead to the total destruction of roots which were apparently healthy and sound when placed in storage.

INOCULATION EXPERIMENTS ON GROWING BEETS

Evidence that the infection takes place only in the seedling stage or through the leaves in old age is found in the results of inoculation experiments made with cultures of *Phoma betae*. In August, 1912, 100 apparently healthy beets growing in the field at Madison, Wis., were inoculated. A portion of the infections were made directly upon the uninjured crown with vegetative cultures upon beet blocks. Other inoculations were made through wounds near the surface of the soil and

still others through wounds on the petiole or in the heart of the crown. In no case was there any evidence of rot in the field or at the time of harvest. It was at first believed that climatic conditions were responsible for the failure. However, the beets were placed in storage. The following spring they were examined for evidences of *Phoma betae* by culture methods. None of the beets had been destroyed; indeed, most of them appeared to be perfectly sound until they were opened, when the black lines previously mentioned appeared in the vascular regions. Cultures were obtained from each of the infected beets, except two. These results were at first interpreted as an indication that the inoculations made in August had produced infection which had persisted on the surface and penetrated the roots in storage, but subsequent developments throw doubt upon this conclusion and indicate that the infection occurred much earlier. About half a ton of beets grown as controls had been placed in storage beside the infected material, but in a lower rack. Evidences of rot were observed in only half a dozen of these beets, and they yielded cultures of *Phoma betae*. It was noted at the time of examination that the control beets, which had been kept nearer the surface of the soil, were all firmer than the inoculated material; but unfortunately no significance was attached to this fact at the time, and, with the exception of 12 of the control beets, the entire lot of material was sent to the feeding sheds.

A few weeks later a large quantity of mother beets grown in the vicinity of the experimental field from western seed was found to be seriously injured by the Phoma rot, practically every beet showing more or less evidence of it. These beets had been bored for analysis in the fall, so that any evidence of infection at that time would have been apparent. The decay did not originate from infection in the wound made by boring, as may be seen readily by reference to the illustration (Pl. XIX). In the large majority of cases the rot evidently started from the crown, although there were cases of pockets of decay on the sides or even at the tip of the beet. In some instances dark streaks could be traced from the crown to decayed areas in the lower portion. This led to the belief that the decay in beets inoculated with *Phoma betae* was due to infection occurring in the seedling stage and that its development was fostered by the less favorable, dryer conditions of storage to which they were submitted. Additional evidence for this belief is to be found in the results of the inoculation experiments with Rhizoctonia (p. 153), since the Phoma rot appeared upon this material also. Moreover, of the few control beets saved from the feeding sheds all but three developed Phoma decay during the late spring and early summer, and cultures of *Phoma betae* were secured from darkened bundles in the crowns of these three. It might be urged that the infection reached the crowns of these beets by way of the petioles from Phyllosticta upon the leaves. This seems a very probable means of infection, but appears unlikely in the instances above cited,

since Phyllosticta spots were at no time observed upon the foliage of the beets in question, which were grown in isolation and were under daily observation. The following season (1913) inoculations were made on beets grown outdoors at Madison, Wis., in pots filled with soil from Garden City, Kans., from Rocky Ford, Colo., and from Madison, Wis. Other beets grown in the usual way in the field at Madison, at Garden City, and at Rocky Ford were inoculated. In all cases this was done by placing a large fragment of actively growing culture on sterilized beet blocks upon the crown of the beet near the heart and a second portion in direct contact with an abrasion on the crown just beneath the surface of the soil. Through the courtesy of colleagues at Garden City and Rocky Ford it was possible to have the beets watched at these points as carefully as were those at Madison. At no time during the season did evidence of infection appear on any of the beets under observation.

Two lots of beets were grown in 1913 from pasteurized seed. One lot was isolated from other beets so as to be protected from infection. These beets were stored in the fall in a warm basement, which offered very favorable conditions for the development of Phoma storage-rot, as shown by previous experience. They were examined from time to time during the winter, spring, and summer for evidences of *Phoma betae*, with negative results.

The other lot of seed was sown in proximity to breeding stock and mother plants known to be infected with *Phoma betae*, and stored in a cellar with infected material. These beets developed Phoma rot during the winter. Phyllosticta appeared on seed plants grown from some of these roots during the summer of 1914, and the seed produced was found to carry the pycnidia and spores of Phoma.

PERPETUATION OF THE FUNGUS

The results of the various experiments coupled with field observations lead to the opinion that *Phoma betae* is capable of infecting *Beta vulgaris* L. only during periods of especial susceptibility, such as the early seedling stage, or, in the case of the leaf, during physiological old age. It may also be that the root is susceptible to infection during periods of low vitality induced by unfavorable environment, but experimental proof is lacking. It does appear, however, that this fungus is entirely capable of living for a long time in a hidden condition upon the crown of the beet, ready to take advantage of any diminished vitality in its host. After infection has once occurred, the parasite may remain present in a viable though inconspicuous condition, although the host appears to have completely overcome the disease. The Phoma field-rot in the summer and fall of 1913, in Wisconsin and Colorado, respectively, developing in the first instance under conditions of excessive moisture and in the second under severe drought, may be explained in this way. The crown

infections originating from the seed readily explain the source of the spores producing the outbreaks of Phyllosticta, which appear to be much more common in regions of low humidity, where, as Pool and McKay (34) have shown, viable spores of this fungus are common in the air. In this connection, attention should be especially called to observations during the seasons of 1912 and 1913 in Colorado, Idaho, and Wisconsin, since they indicate a direct connection between the occurrence of Phyllosticta on the leaves and seed stalks of mother beets and the presence of Phoma on the seed they produce. Phyllosticta was quite prevalent in the Idaho-Colorado region, but escaped detection by the same observer in Wisconsin. Tests upon seed produced in these regions during both seasons have shown that the more western seed was quite generally infected to an extent comparable to that from European sources, while that grown in Wisconsin showed only a very slight infection.

Pool and McKay, who kindly continued the observations at Madison during the absence of the author in the season of 1914, found the Phyllosticta form on the leaves and stalks of seed beets and also on first-year beets. In the absence of fruits the causal fungus was identified by cultural methods. In other instances pycnidia were produced. The spots were, as a rule, less clearly defined and less numerous than those in the Idaho-Colorado region, which are similar to those seen in Europe in 1914, where they were common on both field and mother beets. Since the leaf form of the disease occurred in Wisconsin in 1914, it is probably safe to assume that it existed there on seed beets, undetected, in the two preceding years. However, if the absence of Phyllosticta infection in Wisconsin in 1912 and 1913 be assumed, the presence of the small amount of *Phoma betae* on the seed produced there may still be accounted for in at least three different ways. The seed balls may have been infected at any time between blossom and maturity by air-borne spores developing in the crown. The fungus may have spread from the crown of the mother plants on the surface of the seed stalks to the racemes and infected the fruit, or it may have passed through the vascular system of the stem to the seed.

CONTROL OF THE FUNGUS

It is apparent that *Phoma betae* is one of the most serious obstacles which the growers of beet seed in America have to face. Its ravages on mother beets during the winter may be largely overcome by favorable conditions of storage, but in case the mother beet escapes destruction the infection remains to proceed to the leaves and seed stalks and to infect the seed. Since the fungus sometimes inhabits the vascular region of the root, it may possibly progress through the stem, as well as on the surface. In any event, it is an undoubted fact that it finds its way into the young seed ball, where it starts with the seed upon another 2-year cycle.

From what has been said regarding sources of infection, it appears that ordinary attention to rotation should eliminate danger from soil infection, but that all seed at present available is heavily infected. The only hope of control therefore lies in one or more of three alternatives: (1) The natural resistance of the beet to the attacks of the fungus, (2) seed treatments, (3) the production of disease-free seed.

From the facts that the period of infection with *Phoma betae* is normally confined to a relatively short period in the seedling stage and that infected plants frequently throw off the attack, proper attention to cultural conditions would seem to offer hopeful prospect of control. European experience has demonstrated the value of such methods. Indeed, some prominent agriculturists have denied the pathogenicity of *Phoma betae* because of their success in preventing damping-off and root sickness by proper cultivation and fertilization. From this they have argued that the cause lies in unfavorable cultural conditions rather than the presence of parasites. The truth undoubtedly is that unfavorable environment is a predisposing cause which so weakens the beet that it is unable to compete successfully with the fungus. When planted in good soil, which has been well prepared and suitably fertilized, the seed germinates promptly and the young plants pass rapidly through the period of danger. Early cultivation at this stage to insure proper aeration of the roots is beneficial. The studies upon control by means of cultural methods and fertilizers demonstrate the value of properly prepared soil and thorough cultivation promptly after the seedlings come through the ground. There is no doubt that frequently an infected stand may be saved in this way. In Europe the use of phosphoric acid and potash has given good results. The question of soil reaction has also been found very important. Applications of lime on certain types of acid soil result in almost complete control. This may also prove to be the case in America, but the point can be determined only by local experiment.

As has already been said, the only method of seed treatment which has given satisfactory results in pot experiments is seed pasteurization. Experimental work to test the efficiency of this method in field practice has been attempted. It is not believed that a method of treatment as difficult to carry out as pasteurization will prove useful except in the hands of experimentalists and for experimental purposes. It is apparent that seed must be treated in small lots and with extreme care in order to secure the desired result. If temperatures much above 60° C. are employed, the injury to the seed becomes serious. Temperatures below 60° are ineffective. The substitution of one treatment for two is likewise unsuccessful. Attempts to apply the treatment to samples of seed of even 5 pounds have not been altogether satisfactory, but appear to hold the fungus in check.

One experiment in seed treatment carried out under field conditions where epidemic development of root sickness has annually occurred for

several years will illustrate the point. A half-acre field was prepared. Five pounds of pasteurized seed were sown on one quarter-acre and five pounds of untreated seed on the other. As soon as the seedlings were well out of the ground, examination was made for root sickness and damping-off. No signs of damping-off were seen, but considerable root sickness was in evidence on both the treated and untreated sections. Cultures were started in the field by treating the seedlings for one minute in a triturate of citric acid and bichlorid of mercury in water of such strength as to give a 1 to 1,000 solution of sublimate. They were then transferred to test tubes of sterilized water and were brought to the laboratory, where they were plated. A period of 36 hours elapsed between the treatment with the bichlorid of mercury and the plating. From the untreated seed 95 seedlings yielded the following results: Phoma, 29; Fusarium, 19; Macrosporium, 2; Mucor, 6; miscellaneous, 18; no growth, 21. From the treated seed 69 seedlings yielded: Fusarium, 23; an unidentified ascomycete, 11; Macrosporium, 2; Mucor, 2; Penicillium, 1; miscellaneous, 8; no growth, 22. *Phoma betae* was not found in the treated lot. The long interval before plating undoubtedly accounts for the large amount of Fusarium and perhaps also for the failure to secure growth in many cases.

The average results secured from prompt plating may be seen from the following series, which was from the same locality but was made two weeks earlier. Many of these were plated immediately. Most of the Fusarium resulted from seedlings which were carried in the water blanks for half a day or more. Two hundred seedlings yielded cultures as follows: Phoma, 149; Fusarium, 29; Pythium, 3; miscellaneous, 11; no growth, 8. It therefore appears that, while seed pasteurization may be employed successfully to rid seed of *Phoma betae* for experimental purposes, it is not applicable on a commercial scale. Moreover, such treatment does not guarantee freedom from physiological root sickness associated with saprophytic fungi, since the vitality of the seedlings seems to be lowered by pasteurization.

A realization of these facts suggests the necessity of clean stock for breeding purposes. It would seem that if the growers of elite strains could rid their stock of this parasite it would thereafter remain clean, provided a reasonable rotation were observed and the seed fields were sufficiently isolated to escape reinfection with Phyllosticta. Danger from this source would diminish with the increase in the supply of clean seed. The experimental work already reported has shown the possibility of eliminating the fungus from plants grown in isolation from pasteurized seed. These plants would produce clean seed which could again be sown in isolation from infected stock and made the basis for a seed supply for an entire community, from which the fungus would be eliminated. This community could be employed as a breeding center where the entire seed supply of a factory could be grown, and one of the most

serious fungous pests of the sugar beet eliminated from its territory. Since sugar companies have absolute control over the sources of seed supply of their growers, it is quite possible for a company producing even a portion of its own seed to maintain an area of quarantine within any portion of its territory where it does not compete with other companies for acreage, provided table beets and mangel-wurzels are not allowed to bring in the infection.

RHIZOCTONIA

The genus Rhizoctonia has been used to include a group of sterile fungi, more or less closely related morphologically. Much confusion exists regarding the identity of the various forms, and there is likewise great diversity of opinion as to the pathogenic properties of the members of the group. To make clear, especially to foreign investigators, the identity of the fungus under consideration in this paper, a brief discussion of the literature seems essential. The name "Rhizoctonia" was first applied by De Candolle (8) in 1815 to a fungus on alfalfa. He eventually distinguished three species, *R. crocorum*, *R. medicaginis*, and *R. mali*. During the following 35 years various workers described a series of diseases caused by similar fungi, which were referred to this or other genera. In 1851 Tulasne (43, p. 188) united the known forms of Rhizoctonia into one species under the name "*Rhizoctonia violacea.*" This classification has been followed by many workers.

In 1858 Kühn (27, p. 222–249) published an account of three species, *R. solani*, *R. medicaginis*, and *R. crocorum*. He distinguished between the two forms first mentioned by the difference in appearance of the sclerotia, those of *R. solani* being smooth, and those of *R. medicaginis*, woolly. He mentions *R. medicaginis* as being parasitic on the beet and carrot, as well as alfalfa, and states that the fungus produces a reddish brown or purplish color in the cells of the beet. This is the first mention of Rhizoctonia on the beet, and it is likewise the first mention of the fungus in Germany. Saccardo has included Kühn's species under *R. violacea*, while Güssow (21), who described a disease on potatoes and alfalfa in England due to Rhizoctonia, considers *R. solani* Kühn to be identical with *R. violacea* Tul. Eriksson (14) in 1903 published the results of inoculation work on various hosts, including the sugar beet. He designated his fungus as *R. violacea*, but later reclassified it as *Hypochnus violaceae* (Tul.) Eriks. (15, p. 421–430). He believed there were biological forms of it, since the form on carrot attacked the beet with more virulence in the second generation than in the first in which it was carried on that host.

Atkinson (1) in 1892 described a damping-off of cotton due to a sterile fungus later classified as Rhizoctonia. Balls (2, 3) has found the same disease in Egypt, and Shaw (42) has more recently reported it from India. In the meantime Duggar (10, p. 344) described the same fungus as a damping-off parasite of the sugar beet, and Pammel (30)

reported a root-rot of beets in Iowa that he believed to be due to *Rhizoctonia betae* Kühn. The fungus, however, appears to be indistinguishable from the one which Atkinson and Duggar, respectively, had reported as a damping-off agent on cotton and sugar-beet seedlings and from forms of Rhizoctonia upon a variety of hosts throughout the United States, acting either as damping-off agents or as the causes of other forms of plant diseases. Rolfs (38, 39), working with the fungus on the potato in Colorado, found a fruiting stage which he designated as *Corticium vagum* B. and C., var. *solani* Burt. He was unable to produce the Corticium in culture, but the growth from spores yielded typical Rhizoctonia mycelium, and infections on living plants with Rhizoctonia gave rise to Corticium. Shaw observed the fruiting stage on the groundnut and later succeeded in producing it by artificial infection on that host. European workers have referred what appears to be the same basidial form to *Hypochnus solani.*

Shaw (42) found marked differences in the character of sclerotia produced by his strains. One which formed small black sclerotia more or less differentiated into cortex and medulla he designated as *R. solani* Kühn. The structure of the sclerotia of the other Rhizoctonia which he designated only as *Corticium vagum* B. and C. appears to correspond closely with that of those obtained in America in cultures and less frequently on the host. He believed this Corticium to be identical with the form common on potatoes in America, but was unable to see justification for referring it to *R. solani* Kühn. Furthermore, he believed *R. violacea* Tul. to be a compound species, possibly including *R. solani* Kühn and the Corticium, since Prillieux (36, t. 2, p. 144) described *R. violacea* Tul. as possessing two distinct forms of sclerotia, one of which, according to Shaw, is similar to those of *R. solani* Kühn and the other to *Corticium vagum.*

A form of crown rot on the sugar beet caused by *Rhizoctonia violacea* Tul. is well known in certain sections of Europe, but rot from *Rhizoctonia solani* is unknown there. This fact has led many students to question the identity of the fungus causing the rot of the beet in America. The organism here considered is distinctly different from the *Rhizoctonia violacea* Tul. type as the writer saw it in Europe on living or preserved material from a variety of hosts, including beets, carrots, potato tubers, alfalfa, and asparagus, but it appears to be identical with the *Rhizoctonia solani* type which forms the sclerotia on the potato in Europe and America. These two fungi when studied on the same host differ in the character of the disease produced, in their appearance on the plants, and in the histological relation of the parasite and host. The two are so distinct in these characters on both the beet and the potato that it seems impossible for one who has seen both types to confuse them. The cultural relations of the two are also distinctly different. *Rhizoctonia solani* is readily cultivated on a variety of media, but all attempts to put

R. violacea into artificial culture have thus far failed, though many different workers have undertaken it. So far as can be determined from the literature, the American fungus also appears to be identical with Shaw's (42) *Corticium vagum* B. and C., and it is indistinguishable in culture from a strain isolated in Ireland by Pethybridge from a single spore of Hypochnus on the potato, and kindly contributed by him under the name "*Hypochnus solani*."

The fungus is characterized by a septate mycelium the branches of which in young cultures are either parallel to or inclined at a more or less acute angle to the direction of growth of the parent branch. There is a constriction where the branch unites with the old hypha and a septum is formed a few microns from the point of origin. The threads are hyaline when young, becoming a yellowish brown with age. In mature cultures the branches are usually arranged very nearly at right angles to the parent thread at the point of origin. In culture and less frequently upon the host it forms sclerotia, which vary greatly in size (Pl. XX, fig. 2). One shown in the illustration of a sugar beet measured a full half-inch (Pl. XXIII). They are usually much smaller, from 1 to 3 mm., and those produced in cultures are likely to be quite irregular in outline. The sclerotia consist of interwoven branches, forming a loose pseudoparenchyma of uniform structure throughout. The sclerotial hyphæ are broken up into short cells each of which may function as a spore when placed under favorable conditions for development. The Corticium stage has not been observed upon the sugar beet, but the fungus appears to be identical with the form on the potato and a variety of other plants upon which the Corticium is common, and the name "*Corticium vagum* B. and C., var. *solani* Burt.," which is the one most generally accepted in America, is being retained for the purposes of this paper.

The beet diseases produced by this fungus in America are unknown in Europe, and this fact has been used as an argument that they can not be correctly attributed here to the fungus which produces the sclerotia on potatoes there. This argument is fully met, however, when we approach a study of the environmental factors upon which the fungus is dependent for the production of disease, since the climatic and soil factors under which it becomes an active parasite in some portions of America are not found in Europe.

INOCULATION EXPERIMENTS ON BEET SEEDLINGS

The 34 cultures used in the inoculation experiments on seedlings were obtained from the following sources:

Sugar-beet seedlings grown in the field at Rocky Ford, Colo., 5; at Madison, Wis., in Rocky Ford soil, 5; at Madison in Garden City, Kans., soil, 2; at Washington in greenhouse soil, 3; in sterilized soil infected with decayed beets, 2; in Madison greenhouse soil, 1; at Madison in

field soil, 1; crown-rot sugar beets from Rocky Ford, Colo., 5; from Garden City, Kans., 2; from Chino, Cal., 1; from Kenosha, Wis., 1; potato tuber from Carbondale, Colo., 1; radish from Madison, 1; carrot from Madison, 1; pine seedlings grown at Garden City (contributed by Mr. Carl Hartley, of the Bureau of Plant Industry), 2; and decaying tomato grown on Potomac Flats, Washington, D. C. (contributed by Dr. H. W. Wollenweber (44), of the Bureau of Plant Industry, as *R. potomacensis* Wollenw.), 1.

The various strains in cultures exhibited no striking differences. Those which did appear are due largely to difference in vigor. The virulence is reduced temporarily by long continuance in artificial culture. Difference in the virulence of the several strains, both when freshly isolated and when rejuvenated, was sometimes noted, and this difference appeared to be quite constant, although it bore no relation to the host which furnished the original culture. For example, one of the two strains most virulent to beet seedlings was secured from the beet root and the other was the form from tomatoes received as *R. potomacensis* Wollenw. Certain of the strains that were least virulent were obtained originally from sugar-beet seedlings.

The inoculations were carried out with extreme care, following the methods already described. Every precaution was taken to insure the accuracy of the results, which were uniformly positive. Each strain was recovered and reinoculated into seedlings through from four to six generations.

The type of disease produced upon beet seedlings is similar to that caused by *Phoma betae*, but the plants are attacked at a younger stage, and the progress of decay is likely to be more rapid, so that it was necessary to exercise considerable care in making inoculations at the time of seeding. In the cases of heavy inoculation few or no seedlings broke through the soil. With lighter inoculation a milder form of damping-off developed, or the disease took the form of root sickness, in which case a relatively large number of plants eventually recovered. The fungus is capable of attacking its host at any time after germination. Inoculations upon older seedlings also gave positive results. Young beets 4 or 5 weeks old were readily killed by inoculations upon the crown when no wound was made.

DISTRIBUTION OF THE FUNGUS

The distribution of the fungus is very general, but under field conditions damping-off due to Rhizoctonia is far more general in the soils of the semiarid West. Soils brought from western Kansas and Colorado to Wisconsin and placed in pots in the pathological garden yielded a large percentage of damping-off from *Rhizoctonia* sp., while Wisconsin soils in control pots were practically free from the ravages of this parasite. The fungus has been isolated a few times from the Wisconsin beet fields, but it appears to be of little consequence as a beet parasite under Wisconsin conditions. The reverse is true in Colorado and Kansas, where a majority

of the diseased seedlings examined have yielded cultures of Rhizoctonia. The fungus is the cause of a very destructive crown-rot in the West (Pl. XX, XXI, and XXII), where it frequently becomes epidemic. It is not uncommon to see entire fields of 50 or 100 acres practically destroyed in August by root-rot, of which there is no evidence earlier in the season (Pl. XXI, fig. 1). This form of rot is seen only occasionally in the more eastern beet-growing districts, where it appears to be of no economic importance.

CONDITIONS INFLUENCING INFECTION

The controlling influences in the distribution of the Rhizoctonia diseases of the beet may conceivably be associated with the unequal distribution of the fungus or with differences in climate or in soil, or with any combination of these. Some light has been shed upon this point in the course of the inoculation experiments on growing beets. Field inoculations were first made in Wisconsin, using cultures obtained from Colorado and Kansas. The first series was made on August 21, 1912, by placing portions of mycelium upon agar among the heart leaves of beets in the field. The inoculations were made just at dark, and the beet leaves were moistened with water from a sprinkler, in imitation of a heavy dew. The morning of the 22d was cloudy, and a very little rain fell. The weather of the next few days was dry and hot. Examination a few days later showed that infection had occurred in all except one of the 29 beets inoculated. The disease, however, failed to make the progress typical of western conditions. At the time of harvest, October 23, one beet showed no evidence of infection even at the point where inoculation was made and where the original dried culture was clearly seen. Six showed no injury other than slight lesions on petioles such as shown in Plate XXI, figure 2. Five showed old lesions on the crown, but they had entirely recovered. Eleven beets showed so slight evidence of decay that it was observed only on close examination. Five showed clearly defined decayed spots, but even these were restricted in area. One had entirely lost its original crown of leaves, but had formed scar tissue and had developed new leaves from the meristem at the sides. (Pl. XXII, fig. 1.) This was the only beet which had been injured for the commercial market, unless it might be that the sugar content of the others had been lowered.

A second set of inoculations were made on August 28. In this instance 40 beets were inoculated by placing on the crown of each a portion of Rhizoctonia mycelium growing on a sterilized beet block. The results were very much like those of the first series. While the inoculations took in every case, most of the beets outgrew the infection. Five beets were sufficiently injured to be unfit for market, but only one was killed. In these cases of serious infection the progress of the disease corresponds closely to that seen in the West, although it was far

less rapid. The fungus was readily recovered in culture from them. A photograph of the most seriously injured beet taken at the time of harvest on October 23 is reproduced in Plate XXII, figure 2. At that time the scars where the original infection had been produced could be found on all the beets. Some showed small areas of decay, but most of them were practically sound.

A third series of inoculations were made on September 11 through knife wounds near the surface of the ground. These were made in imitation of cultivator injury and were infected by placing a rapidly growing culture of *Rhizoctonia* sp. on a beet block directly in contact with the injured surface. Thirty beets were inoculated; none of them was destroyed. About half of the number healed completely, so as to leave only a local scar at the point of inoculation. The others showed local decay, more or less characteristic of crown-rot. The fungus was readily recovered from several of these. The largest decayed area produced was about 4 inches in diameter. At least half of this beet was still sound.

At the time of harvest the beets which showed no decay were topped to remove the leaves, the crown being left uninjured. They were placed on racks in the vegetable cellar and examined for decay from time to time. On April 3 all but two of them showed evidence of rot, although in most cases a close examination was necessary to discover it. Eighteen were selected and submitted to cultural tests for Rhizoctonia. Out of 68 attempts to isolate the organism 54 yielded *Phoma betae*, 2 failed to develop, and the remaining 12 gave growths of various saprophytes. In no instance was it possible to secure a culture of Rhizoctonia. It was apparent that those beets which failed to develop decay in the fields had entirely thrown off the infection from Rhizoctonia.

In order to determine to what extent this resistance to attack is to be attributed to local conditions of climate or soil, two large lots of soil from seriously infected beet fields, one in Kansas and one in Colorado, were shipped to Madison, Wis. Both types of soil were quite heavily infected with *Rhizoctonia* sp. That from Kansas was a sandy loam deficient in organic matter. It had received generous applications of factory waste lime and was of good mechanical texture. The Colorado soil was of compact structure containing an admixture of clay and fine silt. It was very deficient in organic matter, so that it was quite impervious and lumped badly. These soils were placed in unperforated, unglazed, 12-inch crocks containing cinders at the bottom for drainage, and sunk into the ground out of doors to within 2 inches of the top. Soil from Madison was employed in similar crocks as a control. Six crocks of each soil were sterilized in an autoclave by heating for 12 consecutive hours under 15 pounds' pressure, and six were left untreated. Untreated beet seed which showed remarkably strong germination and less than 1 per cent of infection with *Phoma betae* was sown. Damping-off developed only in the unsterilized soil from Kansas and Colorado. Attempts to

isolate the causal organisms in 27 cases gave the following results: Rhizoctonia, 15; Phoma, 8; Fusarium, 2; Mucor, 1; undetermined, 1. Both of the parasitic forms were isolated from each of the two types of soil developing disease. A good stand, which was thinned to three, four, or, in a few cases, five plants per pot, was secured in each crock, however, in spite of damping-off. Early in July evidences of crown-rot developed in four of the six pots of unsterilized Colorado soil, but not in that from Kansas. In two of these pots the stand was entirely destroyed by July 21, and in a third there remained only one small seedling with four leaves, which appeared after the original stand had been killed. No disease appeared in the pots of sterilized soil, nor did root-rot develop in the unsterilized Kansas soil. On July 23 inoculations were made with a recently isolated Kansas strain of Rhizoctonia in two pots of each of the six classes of soils. Two pots of each class were reserved as controls, and two were inoculated with *Phoma betae*, as previously reported. The inoculations were made on one beet only in each pot, by placing a piece of beet-block culture on the crown and a second fragment against a wound just below the surface of the soil. The other beets in the pots were not disturbed in any way. One beet in each control pot was wounded in a manner similar to that employed in the inoculations.

The results in inoculated and in uninoculated pots are given in Table V. It is worthy of note that as a result of inoculating 1 beet in each of 12 pots, 26 beets were killed and 7 more were so seriously diseased as to be made worthless, while only 3 resisted infection. One beet attacked in July by spontaneous rot recovered later. The fungus was recovered in culture from several of the diseased roots.

TABLE V.—*Results of pot experiments with Rhizoctonia rot*

INOCULATED POTS

Source of soil.	Pot No.	Number of beets in pot on July 21.	Condition on October 18.		
			Number dead.	Number infected but living.	Number sound.
Colorado................................	19	3	3	....	....
Do....................................	22	a 1	....	1	....
Colorado (sterilized)..................	1	4	3	....	1
Do....................................	4	4	2	2	....
Kansas.................................	25	3	2	1	....
Do....................................	28	4	2	1	1
Kansas (sterilized)....................	31	2	2	....	....
Do....................................	34	4	4	....	....
Wisconsin..............................	13	2	1	1	....
Do....................................	16	3	1	1	1
Wisconsin (sterilized).................	7	3	3	....	....
Do....................................	10	3	3	....	....
Total.................................	....	36	26	7	3

a The original stand of three beets had already been destroyed by spontaneous Rhizoctonia rot. The one plant living resulted from seed delayed in germination.

TABLE V.—*Results of pot experiments with Rhizoctonia rot*—Continued

UNINOCULATED POTS

Source of soil.	Pot No.	Number of beets in pot on July 21.	Condition on October 18.		
			Number dead.	Number infected but living.	Number sound.
Colorado............................	20	....	....	....	....
Do..............................	23	[a] 5	1	....	[b] 5
Colorado (sterilized)..................	2	4	....	....	4
Do..............................	5	4	....	....	5
Kansas..............................	26	3	....	....	3
Do..............................	29	3	....	....	3
Kansas (sterilized)...................	32	2	....	....	2
Do..............................	35	3	....	....	3
Wisconsin...........................	14	4	....	....	4
Do..............................	17	3	....	....	[b] 4
Wisconsin (sterilized)................	8	3	....	....	3
Do..............................	11	3	....	....	3
Total........................	....	37	1	....	39

[a] Two of these beets were diseased with Rhizoctonia rot. One of them eventually recovered, and the other died.
[b] The additional beet is known to have resulted from seed delayed in germination.

Other inoculations with Rhizoctonia were made on beets growing in the field at Madison, Wis., Garden City, Kans., and Rocky Ford, Colo.

Of 30 plants inoculated at Madison on July 23, 1 escaped infection, 2 were infected but recovered, and 27 were killed. The fungus was recovered in culture from several of them. The control plants, of which there were several hundred, remained healthy.

The inoculations at Garden City were made on July 27 on beets furnished by Dr. C. F. Clark, of the Bureau of Plant Industry, who kindly made the field observations. The field was known to be somewhat infected with Rhizoctonia. One row was inoculated, those adjacent on either side being reserved as controls. The same procedure was observed in the inoculations with *Phoma betae*, previously reported, but since these did not produce disease, five rows, or 150 beets, became available for controls. Of the 30 inoculated plants, 23 were killed, 2 others were so seriously injured at the crown as to become entirely defoliated and apparently dead but developed a few new leaves late in the fall (October), and 5 escaped infection. The rate of progress of the disease is shown in Table VI. Three control beets became infected during the season.

TABLE VI.—*Results of inoculation experiments with Rhizoctonia sp. at Garden City, Kans.*

Row No.	Treatment.	Number of dead sugar-beet plants on—						Total number of dead plants.	Number of healthy plants on Sept. 23.
		Aug. 7.	Aug. 13.	Aug. 23.	Sept. 3.	Sept. 17.	Sept. 23.		
1	Control............	0	0	1	0	0	0	1	29
2	Rhizoctonia.....	2	1	11	6	1	4	[a] 25	5
3	Control...........	0	0	0	0	0	1	1	29
4	Do..........	1	0	0	0	0	0	1	29
5	*Phoma betae*.....	0	0	0	0	0	0	0	30
6	Control..........	0	0	0	0	0	0	0	30

[a] Two of these made feeble effort at recovery in October, showing that a little parenchyma had survived.

The 30 beets inoculated at Rocky Ford were killed, while all the controls remained healthy (Pl. XX, fig. 1).

A consideration of the facts related indicates that soil properties are potent factors influencing the susceptibility of beets to attack by Rhizoctonia. It has long been maintained that clay soils and those which are seriously deficient in organic matter and of fine, compact texture, so as to bake readily, are most likely to develop Rhizoctonia diseases. Further indication of this is found in the development of spontaneous root-rot in the Colorado soils used in the pot experiments at Wisconsin. It did not develop in the other soils employed, although the amount of Rhizoctonia damping-off indicated that the Kansas soil was at least as heavily infected with Rhizoctonia as was the Colorado material. The experimental data lead to the conclusion, however, that, in the case of the Rhizoctonia root-rot of the beet, soil temperature is a more important factor than soil texture. The inoculations in the field at Madison in 1912 were made at the beginning of a very hot period which endured for several days. Infection was produced uniformly, but in practically every case the beets completely recovered during the cooler weather which set in a few days after the inoculations were made. In 1913 infection was attempted at an earlier date when it might be expected that a somewhat longer period of hot weather would ensue. This proved to be the case and, as already pointed out, the inoculations were very generally successful. It is also significant that the cases of partial or complete recovery which occur appear late in the season when the soil temperatures are considerably lowered.

PYTHIUM DEBARYANUM

Hesse (24) reported *Pythium debaryanum* Hesse as a damping-off parasite of beets in 1874, but his experimental work appears to have been done on other hosts, so that while no one has doubted the accuracy of Hesse's deductions, Peters (33, p. 221) appears to have been the first to demonstrate by culture methods that this fungus includes the sugar

beet among its hosts. The fungus is so well known that a description here is superfluous. It is very readily secured in pure culture and is easily carried upon media (Pl. XVI, fig. 1). It grows especially well with long-continued vitality upon string-bean agar. The sexual fruiting bodies are quite common in Petri-dish cultures upon this medium, but are rarely met with in tube cultures. The asexual conidia, as well as oospores, are formed abundantly when the fungus is grown in water upon sugar-beet seedlings in Petri dishes. The cultures obtained throughout the experiments were invariably identified by fruiting bodies, and the same method was applied in proving up the cultures recovered from artificial inoculation. Suspected seedlings were treated in bichlorid of mercury, rinsed in water, and plated upon the acid synthetic agar previously mentioned (p. 137). When growth developed, the mycelium was examined through the bottom of the Petri dish by inverting the plate upon the stage of the microscope. If no septa were visible, the seedling was transferred to a sterile Petri dish after a subculture had been made from the growth. Sterile water was added to the fresh plate containing the seedling. In case the growth was *Pythium debaryanum*, the characteristic conidia developed in great numbers in from 24 to 48 hours, to be followed during the next few days by oospores. Direct germination of conidia was often seen and could be very readily induced by adding a fresh beet seedling to the culture. Germination by zoospores was not observed, but no special effort was made to induce this type of development.

The cultures used in the inoculation experiments were all morphologically identical, so far as could be determined. They were secured from the following sources:

Damped-off beets grown at Washington in the greenhouse, 2; at Madison, Wis., in the greenhouse, 6; at Madison in the greenhouse in Utah soil, 3; at Madison in the greenhouse in Michigan soil, 2; damped-off seedlings grown in Utah in the field, 1; grown in Wisconsin in the field, 1; grown in Colorado in the field, 1; damped-off pine seedlings from Kansas (contributed by Mr. Carl Hartley, of the Bureau of Plant Industry), 1; decaying potatoes, isolated in 1909, 1.

Mr. Hartley reported this strain pathogenic to pine seedlings, having produced damping-off with it to the extent of 100 per cent in the seed bed.

Pythium debaryanum proved to be exceedingly destructive in the pot experiments. When infection was made at the time of seeding, even a temporary stand was seldom secured. Examination showed that the seed germinated, but that the plants were destroyed before they could come up. In many cases the embryo was killed while still within the seed. By delaying the inoculation until the seedlings were well started typical damping-off was produced and the fungus recovered. It was a very common thing to find infection on the tips of the cotyledons.

This probably occurred while the leaves were still within the seed coat. The fungus was found to be capable of attacking the beet after it was 5 or 6 weeks old. Peters's statement (33, p. 228) that it is able to infect the side roots during the entire vegetative period is probably correct. When the taproot is once attacked by *P. debaryanum*, the ultimate death of the plant seems to be assured. Fortunately the soil relations in early seeding time are usually not sufficiently favorable to the rapid development of the fungus to make it an aggressive parasite under average field conditions. This fungus does not develop well in cold soil, but does its most serious work under seed-bed and greenhouse conditions or in the fields which have been seeded very late when the soil temperatures have begun to rise.

UNDESCRIBED SPECIES INJURIOUS TO SUGAR BEETS

In the author's preliminary note (12) it was reported that *Aphanomyces laevis* De By. had been found as a damping-off fungus of sugar beets in America, but subsequent detailed morphological studies of the fungus as it developed in artificial culture and on beet seedlings have shown that it differs in some important respects from the published descriptions of De Bary and others. *A. laevis* was first reported in a parasitic relation by Peters (31) in 1906. He found the fungus as a damping-off parasite of considerable importance upon sugar beets in Germany. Barrett (4) has reported its occurrence in America as the cause of a disease of radishes. The first cultures of the fungus temporarily mistaken for *A. laevis* were secured from damped-off beet seedlings grown in soil which had previously produced the black-root disease of the radish, like those shown in Plate XXIV, figure 2. It was later obtained from soils at Madison, and from Kenosha, Wis., as well as from seedlings damping-off in soil which had been infected with fragments of a diseased radish obtained from Illinois. The causal relation of the organism to the radish disease as well as to damping-off of sugar-beet seedlings was confirmed repeatedly by inoculation experiments, and it was at first thought possible that the discrepancies between this fungus and published descriptions of *A. laevis* might be the result of response to the changed environmental conditions of culture or to variations within the species, since it is well known from the study of many investigators that the Saprolegniaceae are exceedingly variable. Through the courtesy of the Kaiserliche Biologische Anstalt at Dahlem, Germany, and Dr. Leo Peters, of that institution, the author was permitted to isolate Aphanomyces from the experimental fields of the Anstalt. An organism was secured from damped-off seedlings, which Dr. Peters identified as the organism with which he had worked and which conformed in every respect to De Bary's description of *A. laevis*. It was secured in pure culture, and its pathogenicity to beet seedlings was confirmed by inoculation experiments. Unfortunately the culture was lost

before it had been tested upon the radish and thereafter could not be secured again. The morphological studies, however, prove that the American fungus with which we have been working is not *A. laevis*, but a hitherto undescribed organism. Morphological, physiological, and cytological studies will be presented in another paper.

In the work with sugar-beet seedlings five strains of the organism were employed which were obtained from the following sources:

Seedling sugar beets grown at Madison, Wis., 2; at Kenosha, Wis., 1; in soil originally infected by radishes showing black-root, 2.

The disease which it produces on the sugar beet is very similar to that caused by *Pythium debaryanum*. The fungus is even more aggressive as a parasite than Pythium (Pl. XXIV, fig. 1). When the inoculations were made at the time of seeding, it was unusual for plants to appear aboveground. The evidence obtained all goes to show that a seedling once attacked never recovers.

A disease of the side roots of growing beets was encountered during the course of the studies in soils which had been inoculated with artificial cultures of the fungus several months earlier. A photograph of a specimen is reproduced in Plate XXIII, figure 2. The fungus was readily isolated from diseased side roots of this beet and there appears to be no reason to doubt its causal relation to the trouble. Peters (33, p. 244) has quoted a similar disease of European beets caused by Aphanomyces.

Comparatively little is known regarding the range of distribution of the fungus. What appears to be the black-root of radish has been observed in the District of Columbia, Maryland, Virginia, Long Island, Illinois, and at several points in Wisconsin, and there is reason to believe that other workers have found it in various places, although no records of such observations have been published. The disease produced by this fungus is so similar to that reported by Barrett (4) that they are not readily distinguishable, and it may be that either of these organisms is responsible for the disease in any of the stations mentioned. The author does not consider that his results should be construed to throw doubt upon the accuracy of Barrett's observations, merely wishing to record a radish disease that is indistinguishable in external appearance from that produced by *Aphanomyces laevis*, but which is due to this hitherto undescribed parasite.

The fungus is readily isolated from beet seedlings by the method already described for *Pythium debaryanum*. In this case, however, the subcultures should be made to string-bean agar, upon which the organism produces a luxuriant growth. Sterilized beet seedlings in water in test tubes make an excellent medium for the cultivation of this fungus. The limit of vitality in culture has not been determined, but transfers made to string-bean agar on July 2 from beet-seedling water cultures made on February 12 developed a heavy growth overnight, showing not the least loss of vitality.

The vegetative stage of the fungus is strikingly like that of *P. debaryanum*, so that they can not be distinguished readily except by the fruiting bodies, which develop readily in water cultures in plates in from 24 to 48 hours. The zoospores develop first, to be followed somewhat later by the Pythium-like oospores. The asexual fruiting bodies are first noted as the swollen ends of hyphæ, which vary greatly in length and are characteristically somewhat branched. They average from 150 to 900μ in length or even more. When mature, these bodies discharge their contents in a spherical mass which cleaves in the course of 20 or 30 minutes, giving rise to numerous zoospores.

OTHER FUNGI FOUND ON SUGAR BEETS

In the course of the isolation work various other fungi were secured. Some of these were known saprophytes, while others, like Macrosporium, Mucor, and Botrytis, have sometimes been reported in parasitic relations, but gave negative results in our trials. Fusarium and Verticillium cultures were secured frequently, but inoculation experiments with these genera were deferred pending the completion of taxonomic work by other investigators.

There remains to be discussed a peculiar type of decay of growing beets and a root sickness of seedlings associated with *Rhizopus nigricans* Ehr. Cultures of this fungus were frequently isolated in the course of experimental work from seedlings. Specimens of mature beets affected by a peculiar light-brown decay were received in the laboratory from California during the campaign of 1910. The interior portion of these beets yielded a very large proportion of cultures of Rhizopus. The decay was very characteristic and unlike anything before seen. In the early stages the material was almost normal in appearance, except for the discoloration. It later assumed a somewhat flabby texture and developed pockets in the interior which were filled with a nearly colorless fluid rich in acetic acid, as was determined by the odor and by chemical tests.

In 1912 a somewhat similar trouble was reported from Colorado, and a visit was made to the field (Pl. XXV, fig. 1). The beets at that time were dead over considerable areas. Those most recently attacked showed the same light-brown color previously referred to (Pl. XXV, fig. 2), while those in the more advanced stages of decay presented various symptoms between the early condition and almost complete dissolution of all except the vascular tissue. Some, however, were apparently pickled in acetic acid, formed probably from the fermentation of the carbohydrate content. A very large number of attempts to isolate an organism of known pathogenic properties was made. The trials yielded *Rhizopus nigricans* in pure cultures to the extent of almost 100 per cent. Inoculation work in the laboratory upon dormant beets in moist chambers resulted in the

reproduction of decay similar in appearance to that occurring in the two cases described. The acetic-acid development, however, did not occur except in a slight degree in a few instances, and there is no certainty that in these cases it did not result from contamination. Inoculation experiments made in the field upon living material invariably yielded negative results. Inoculation experiments upon seedlings made in the usual manner also failed to produce damping-off when reasonably good conditions of culture were maintained. It was possible, however, to produce a disease which showed the symptoms of root sickness when the soil was excessively wet and the temperature rather adverse. Naturally the fungus was easily isolated from such material. The control plants, however, were sickly or diseased on the roots, and it is highly probable that the results obtained in the inoculation experiments with seedlings are to be attributed to physiological injury, which opened the way for the Rhizopus to grow saprophytically upon the tissue.

Inquiry into the history of the fields that produced the peculiar rot with which this fungus was associated revealed the fact that at least one of them had been flooded for a time and that the other had been excessively moist for several consecutive days prior to the appearance of the disease. In view of these facts and the results of experimental work, it seems reasonable to conclude that the beets were originally killed or at least materially weakened by adverse physiological conditions and that *Rhizopus nigricans* followed as a saprophyte or weakling parasite producing a characteristic type of decay.

ALKALI INJURY TO SUGAR BEETS

During a field trip in Colorado late in August, 1912, the writer was called upon to visit a beet field in which a peculiar rot was developing. The beets had made a good growth, and most of them were above the average in size. The foliage was luxuriant, but was characterized by a bluish green color and a brittle texture. Little evidence of disease, aside from the abnormal appearance of the foliage, was evident until the plants were pulled, when it was seen that many beets were decayed at the lower portion of the root. Some agency had killed the taproot, following which a soft rot was destroying the tissue. A majority of the plants in the portions of the field most seriously affected showed a characteristic cracking and corroding of the root, like that shown in Plate XXVI.

Evidences of alkali could be seen on the surface of the soil here and there, and it seemed probable that the deeper branches of the taproot had been killed by alkaline waters. This probability was increased by the fact of the close proximity to the field of an irrigation reservoir the waters of which were evidently quite alkaline, as could be seen from the crust of salts on the ground at the edge of the lake. As a further test upon this opinion, several beets were taken to the laboratory and attempts were

made to isolate pathogenic organisms, but with negative results. Several of the plants upon which decay already had made considerable progress were placed in fresh soil in the greenhouse and held under observation to determine whether the disease would continue to develop. Without exception these plants healed and thereafter showed no evidences of disease. The foliage which they put forth was normal, giving no evidence of the brittleness or blue-green color noted in the field. The results seemed to justify the conclusion that the plants were suffering from excessive alkali brought in by seepage from the neighboring reservoir.

SUMMARY

The more important points brought out in this paper may be summarized as follows: Four fungi have been found to stand in a causal relation to damping-off of sugar beets in America. These are *Phoma betae* (Oud.) Fr.; *Rhizoctonia* sp. probably identical with *Corticium vagum* B. and C., var. *solani* Burt.; *Pythium debaryanum* Hesse; and an undescribed member of the Saprolegneaceae.

Under favorable conditions of culture, plants attacked by *Phoma betae* or Rhizoctonia may recover either temporarily or permanently. Attacks of the other two fungi upon the seedlings may be expected to prove fatal. Phoma and Rhizoctonia are capable of producing characteristic decay in mature beets. The former appears to infect the plants primarily in the seedling stage, and when recovery occurs it remains thereafter in a dormant condition upon the host. It occasionally develops a characteristic black rot on growing beets in the field and more frequently appears upon mother beets in storage. When it does not destroy the root, it may infect the seed stalk and appear upon the mature seed. Control measures are to be sought in proper cultural methods and seed treatment which looks forward to the production of seed free from infection. *Pythium debaryanum* is capable of attacking the feeding roots of the beet throughout its vegetative period, and the new fungus is also able to cause trouble on mature beets in a similar manner. *Rhizopus nigricans* Ehr., while unable to produce disease on normal plants in the field, is capable of attacking the tissue of dead or dormant sugar beets, producing a characteristic decay.

LITERATURE CITED

(1) ATKINSON, G. F.
    1892. Some diseases of cotton. Ala. Agr. Exp. Sta. Bul. 41, 65 p., 25 fig.
(2) BALLS, W. L.
    1906. Physiology of a simple parasite. Preliminary note. *In* Yearbook, Khediv. Agr. Soc., 1905, p. 173–195, pl. 6–7.
(3) ——
    1907. Physiology of a simple parasite. Pt. 2. *In* Yearbook, Khediv. Agr. Soc., 1906, p. 93–99, pl. 13–16.
(4) BARRETT, J. T.
    1912. A serious root disease of radish. (Abstract.) *In* Phytopathology, v. 2, no. 2, p. 96.

(5) BUSSE, Walter.
    1908. Der Wurzelbrand der Rüben. *In* Bl. Zuckerrübenbau, Jahrg. 15, No. 19, p. 297–300, 1 col. pl.

(6) —————— PETERS, Leo, and FABER, von.
    1907. Über den Wurzelbrand der Zuckerrüben. *In* Mitt. K. Biol. Anst. Land- u. Forstw., Heft 4, p. 15–18.

(7) —————— —————— and ULRICH, P.
    1911. Über das Vorkommen von Wurzelbranderregern im Boden. *In* Arb. K. Biol. Anst. Land- u. Forstw., Bd. 8, Heft 2, p. 260–302.

(8) CANDOLLE, A. P. de
    1815. Mémoire sur les Rhizoctones . . . *In* Mém. Mus. Hist. Nat. [Paris], t. 2, p. 209–216, pl. 8.

(9) DUGGAR, B. M.
    1899. Three important fungous diseases of the sugar beet. N. Y. Cornell Agr. Exp. Sta. Bul. 163, p. 339–363, fig. 49–63.

(10) —————
    1909. Fungous Diseases of Plants. 508 p., illus., pl. Boston and New York.

(11) ————— and STEWART, F. C.
    1901. The sterile fungus Rhizoctonia as a cause of plant diseases in America. N. Y. Cornell Agr. Exp. Sta. Bul. 186, p. 51–76, fig. 15–23.

(12) EDSON, H. A.
    1913. Damping-off and root rot parasites of sugar beets. *In* Phytopathology, v. 3, no. 1, p. 76.

(13) EIDAM, Eduard.
    1888. Untersuchungen zweier Krankheits-Erscheinungen die an den Wurzeln der Zuckerrübe in Schlesien letztem Sommer ziemlich häufig vorkommen sind. *In* 65. Jahresber. Schles. Gesell. Vaterländ. Cult., 1887, p. 261–263.

(14) ERIKSSON, Jakob.
    1903. Einige Studien über den Wurzeltöter (Rhizoctonia violacea) der Möhre, mit besonderer Rücksicht auf seine Verbreitungsfähigkeit. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 10, No. 22/23, p. 721–738, fig. 1–3; No. 24/25, p. 766–775, fig. 4, 1 pl.

(15) —————
    1912. Svampsjukdomar å svenska betodlingar. *In* K. Landtbr. Akad. Handl. och Tidskr., årg. 51, no. 6, p. 410–437, 9 fig.

(16) FRANK, A. B.
    1892. Ueber Phoma Betae, einen neuen parasitischen Pilz, welcher die Zuckerrüben zerstört. *In* Ztschr. Ver. Rübenz. Indus. Deut. Reichs, Bd. 42 (n. F. Jahrg. 29), p. 904–916, pl. 20.

(17) —————
    1894. Zur Bekämpfung von Phoma Betae. *In* Ztschr. Ver. Rübenz. Indus. Deut. Reichs, Bd. 44 (n. F. Jahrg. 31), Tech. T., p. 158–169.

(18) —————
    1895. Neue Untersuchungen über Phoma Betae. *In* Ztschr. Ver. Rübenz. Indus. Deut. Reichs, Bd. 45 (n. F. Jahrg. 32), Tech. T., p. 157–188, 271–293, pl. 9–11 (2 col.).

(19) —————
    1896. Bericht über Versuche zur Bekämpfung der Herz und Trockenfäule der Zuckerrüben im Jahre 1896. *In* Ztschr. Ver. Rübenz. Indus. Deut. Reichs, Bd. 46 (n. F. Jahrg. 33), Tech. T., p. 901–928, 1 fig.

(20) —————
    1898. Ueber die durch Phoma Betae verursachte Blattflecken- und Samenstengel-Krankheit der Rüben. *In* Ztschr. Ver. Deut. Zuckerindus., Bd. 48 (n. F. Jahrg. 35), Tech. T., p. 711–717.

(21) GÜSSOW, H. T.
    1905. Potato scurf and potato scab. *In* Jour. Roy. Agr. Soc. England, v. 66,
        p. 175.
(22) HEDGCOCK, G. G.
    1904. Proof of the identity of Phoma and Phyllosticta on the sugar beet. *In*
        Jour. Mycol., v. 10, no. 69, p. 2–3.
(23) HELLRIEGEL, F. H.
    1890. Welche Bedeutung hat die Schädigung der jungen Rüben durch Wurzel-
        brand (schwarze Beine) und welche Mittel gegen dies Uebel sind
        bekannt? *In* Deut. Zuckerindus., Jahrg. 15, No. 24, p. 745.
(24) HESSE, Rudolph.
    1874. Pythium de Baryanum ein endophytischer Schmarotzer in den Geweben
        der Keimlinge der Leindotter, der Rüben, des Spergels und einiger
        anderer landwirtschaftlichen Kulturpflanzen. 76 p., 2 pl. Halle
        a/S. Inaugural-Dissertation—Göttingen.
(25) KRÜGER, Friedrich.
    1893. Phoma Betae (Frank), als einer der Erreger von Wurzelbrand der Rüben-
        pflanze. *In* Ztschr. Ver. Rübenz. Indus. Deut. Reichs, Bd. 43 (n. F.
        Jahrg. 30), p. 730–743.
(26) ———
    1893. Weitere Untersuchungen über die neue Krankheit der Zuckerrübe
        verursacht durch Phoma Betae (Frank). *In* Ztschr. Ver. Rübenz.
        Indus. Deut. Reichs, Bd. 43 (n. F. Jahrg. 30), p. 90–111, 7 fig.
(27) KÜHN, J. G.
    1858. Die Krankheiten der Kulturgewächse ... 312 p., 7 pl. Berlin.
(28) LIND, Jens.
    1913. Danish Fungi as Represented in the Herbarium of E. Rostrup. 648 p.,
        illus., 9 pl. Copenhagen.
(29) OUDEMANS, C. A. J. A.
    1877. Aanwinsten voor de flora mycologica van Nederland van Juli 1875 tot Juli
        1876. *In* Nederland. Kruidk. Arch., s. 2, deel 2, stuk 3, p. 176–188.
(30) PAMMEL, L. H.
    1891. Preliminary notes on a root-rot disease of sugar beets. Iowa Agr. Exp.
        Sta. Bul. 15, p. 243–254, fig. 1–2, pl. 3–6.
(31) PETERS, Leo.
    1906. Zur Kenntnis des Wurzelbrandes der Zuckerrübe. *In* Ber. Deut. Bot.
        Gesell., Bd. 24, Heft 6, p. 323–329.
(32) ———
    1907. Der Wurzelbrand der Zuckerrüben. *In* Umschau, Jahrg. 11, No. 5,
        p. 85–87, 4 fig.
(33) ———
    1911. Über die Erreger des Wurzelbrandes. *In* Arb. K. Biol. Anst. Land- u.
        Forstw., Bd. 8, Heft 2, p. 211–259, 12 fig.
(34) POOL, Venus W., and McKAY, M. B.
    1915. Phoma betae on the leaves of the sugar beet. *In* Jour. Agr. Research,
        v. 4, no. 2, p. 169–178.
(35) PRILLIEUX, E. E.
    1891. La pourriture du cœur de la betterave. *In* Bul. Soc. Mycol. France,
        t. 7, p. 15–19.
(36) ———
    1895–1897. Maladies des Plantes Agricoles ... 2 t. Paris.
(37) ——— and DELACROIX, Georges.
    1891. Complément à l'étude de la maladie du cœur de la betterave. *In* Bul.
        Soc. Mycol. France, t. 7, p. 23–25, pl. 3.

(38) ROLFS, F. M.
   1902. Potato failures. A preliminary report. Colo. Agr. Exp. Sta. Bul. 70, 20 p., 12 pl.

(39) ———
   1904. Potato failures. A second report. Colo. Agr. Exp. Sta. Bul. 91, 33 p., 5 pl.

(40) ROSTRUP, Emil.
   1889. Oversigt over de i 1888 indløbene Forespørgsler angaaende Sygdomme hos Kulturplanter. Voredrag i det Kgl. Landhusholdningsselskab 20. Marts 1889. *In* Tidsskr. Landökonom., Raekke 5, Bd. 8, p. 744–751. Reprint, Kjøbenhavn, 1890. Original not seen.

(41) ———
   1894. Phoma-Angriff bei Wurzelgewächsen. *In* Ztschr. Pflanzenkrank., Bd. 4, p. 322–323.

(42) SHAW, F. J. F.
   1912. The morphology and parasitism of Rhizoctonia. *In* Mem. Dept. Agr. India, Bot. Ser., v. 4, no. 6, p. 115–153, 11 pl.

(43) TULASNE, L. R., and TULASNE, Charles.
   1851. Fungi Hypogaei ... 222 p., 21 pl. (partly col.) Parisiis.

(44) WOLLENWEBER, H. W.
   1913. Pilzparasitäre Welkekrankheiten der Kulturpflanze. *In* Ber. Deut. Bot. Gesell., Bd. 31, Heft 1, p. 30.

PLATE XVI

Isolation cultures from sugar-beet seedlings.

Fig. 1.—*Rhizoctonia* sp.
Fig. 2.—*Pythium debaryanum*.

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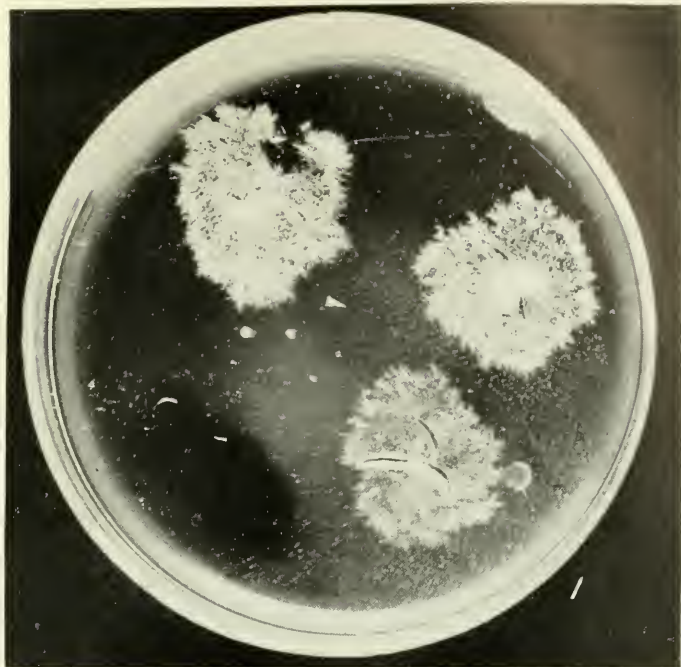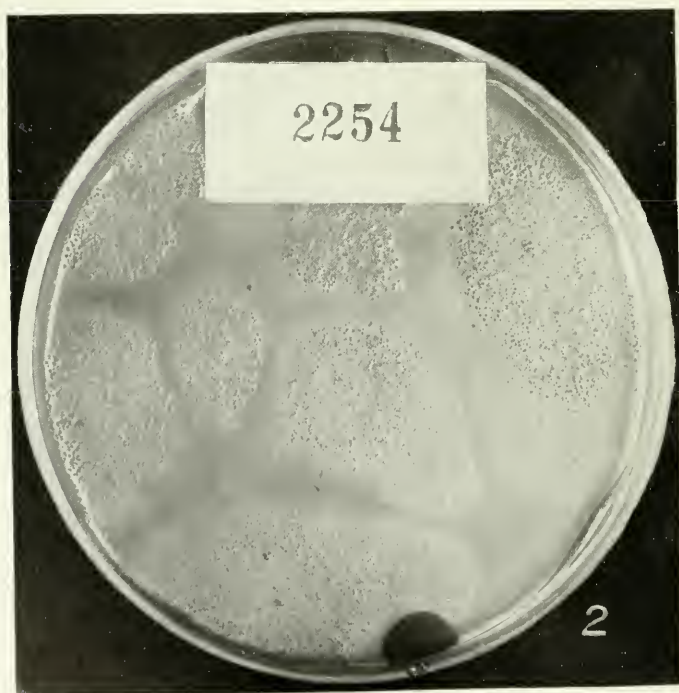
PLATE XVII

Phoma betae.

Fig. 1.—Isolation culture from sugar-beet seedling.
Fig. 2.—Fruiting culture on string-bean agar.

PLATE XVIII

Fig. 1.—Half-grown sugar beets showing crown-rot caused by *Phoma betae*.
Fig. 2.—Sugar beet showing seedling injury caused by *Phoma betae*.

1

2

168 ⁴

PLATE XIX

Mother beet showing storage decay caused by *Phoma betae*.

PLATE XX

Rhizoctonia sp.: Root-rot of sugar beet.

Fig. 1.—Result of artificial inoculation, control beet in center.
Fig. 2.—Result of natural field infection.   Note sclerotia on specimens at right.

2
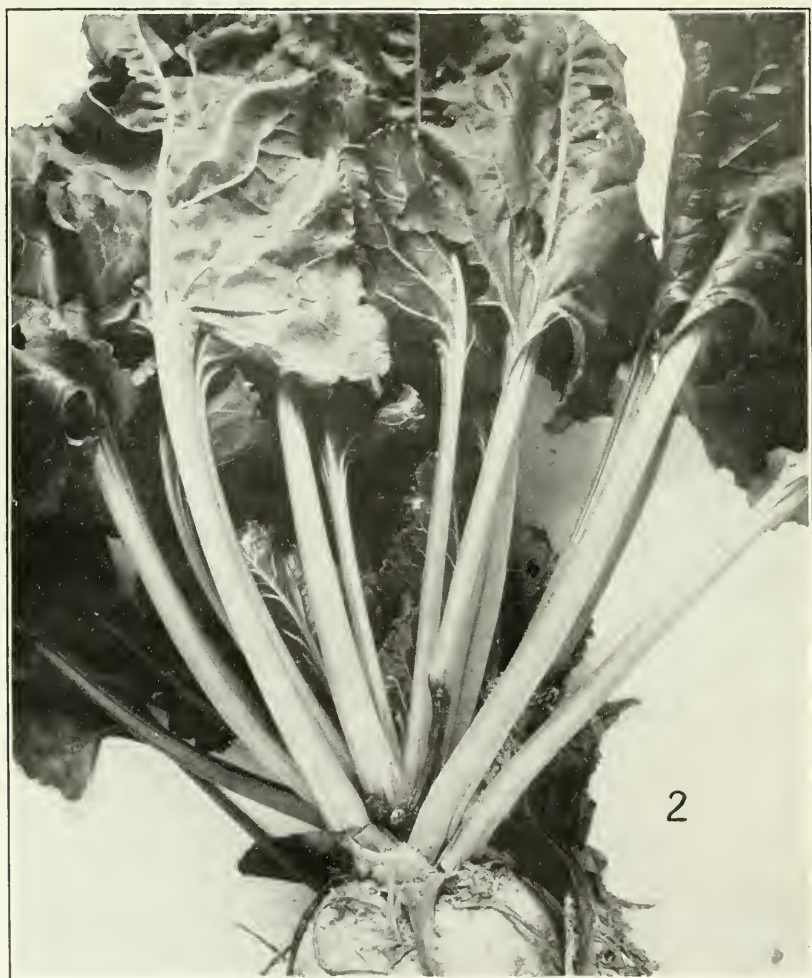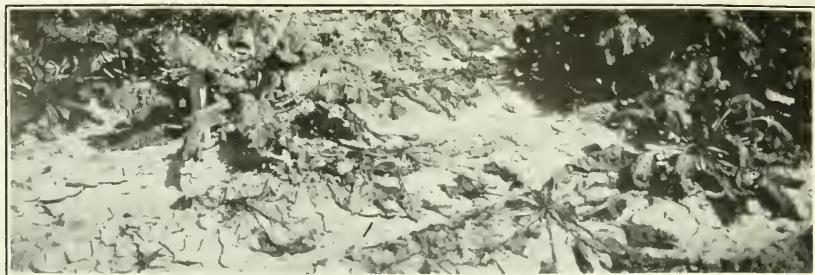
PLATE XXI

Fig. 1.—Sugar beet showing field-rot caused by *Rhizoctonia* sp.   Natural infection.
Fig. 2.—Sugar beet showing artificial infection with *Rhizoctonia* sp. on the petiole.
The disease has been arrested.

Results of artificial inoculation with *Rhizoctonia* sp.

Fig. 1.—Sugar beet, photographed from above, showing original crown destroyed, and new leaves developing from the sides.

Fig. 2.—Section of sugar beet showing character of Rhizoctonia injury.
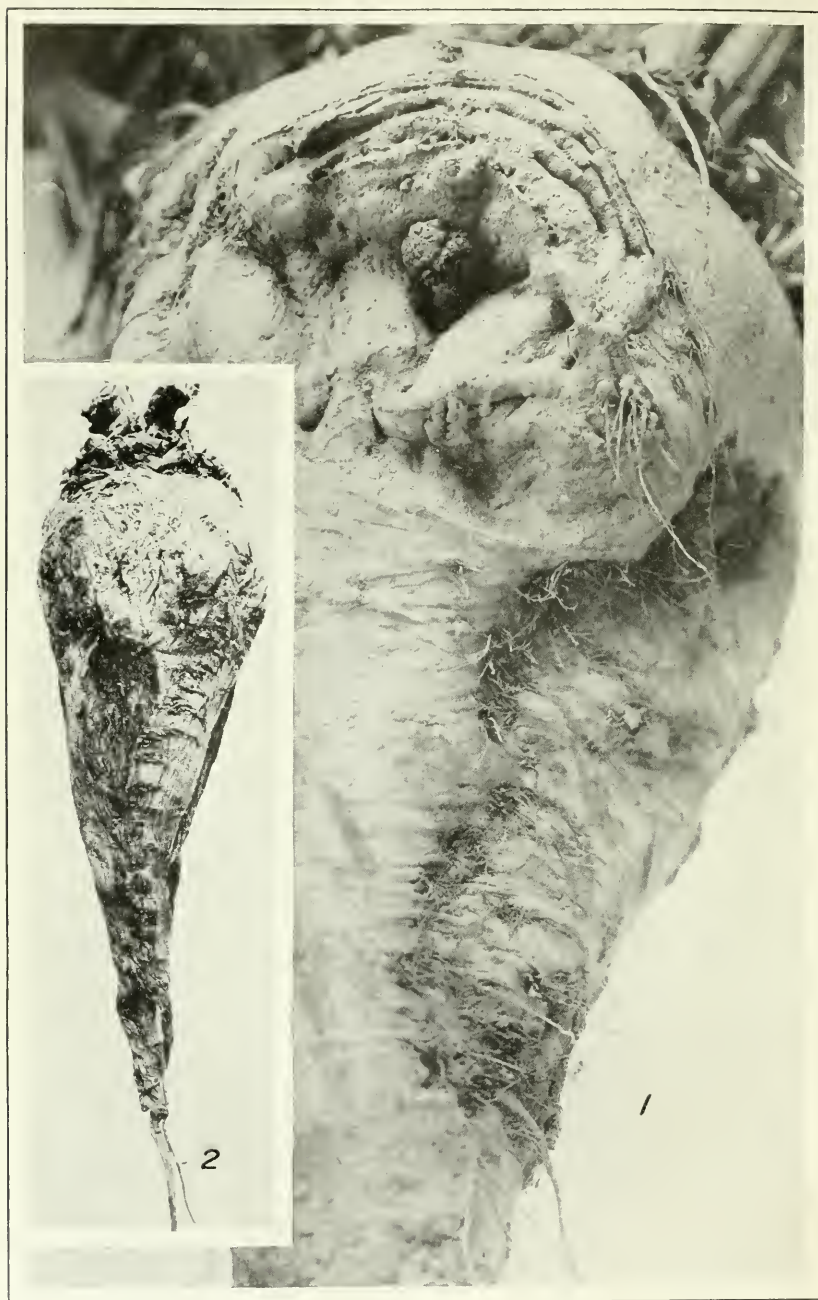
PLATE XXIII

Fig. 1.—Sugar beet showing large sclerotium (½ inch) resulting from artificial inoculation with Rhizoctonia. The beet has resisted infection.

Fig. 2.—Half-grown sugar beet showing injury to feeding roots due to an undescribed parasite.

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PLATE XXIV

Fig. 1.—Sugar beet showing damping-off due to an undescribed parasite; control pot at right.

Fig. 2.—Radish showing black-root caused by the same fungus.

PLATE XXV

PLATE XXV

Fig. 1.—Field in which Rhizopus rot developed.
Fig. 2.—Typical beets from the field shown in figure 1.

PLATE XXVI

Sugar beet showing alkali injury.
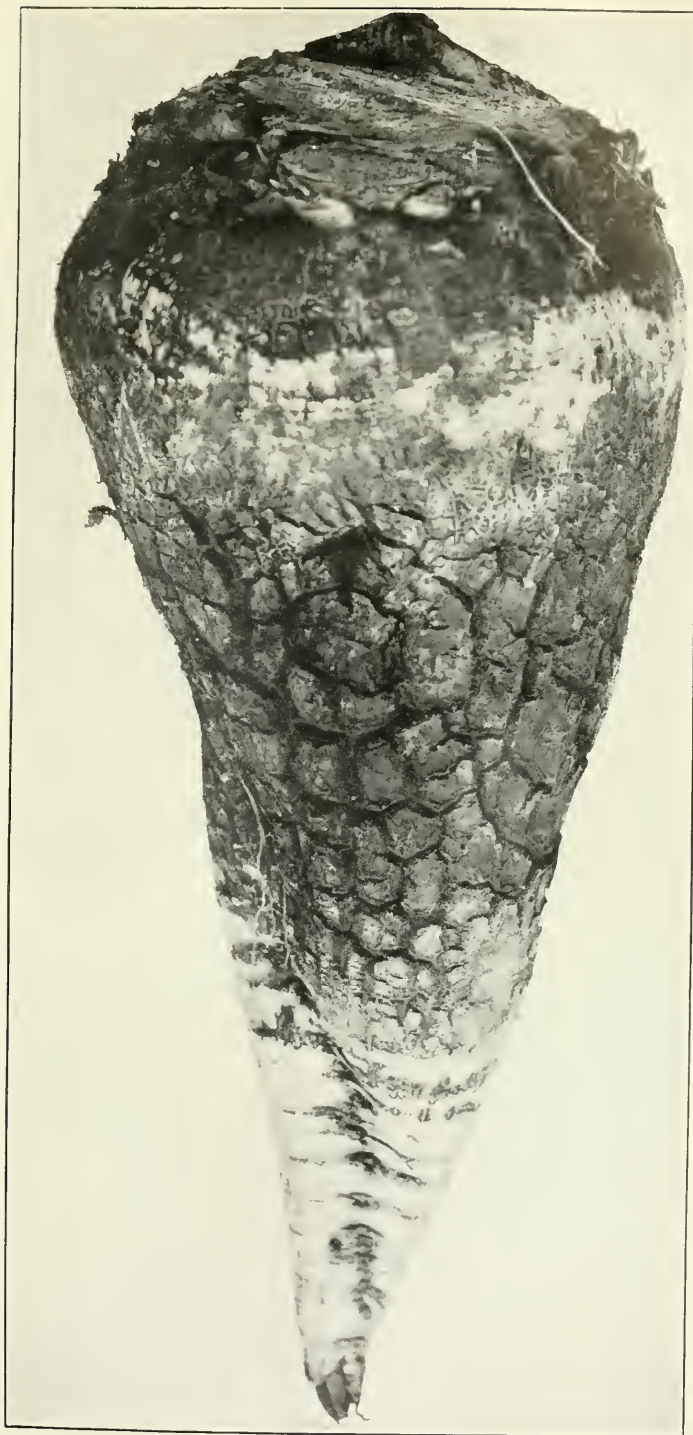
PHOMA BETAE ON THE LEAVES OF THE SUGAR BEET

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INTRODUCTION

Various names have been given at different times to the fungus causing a root-rot, a damping-off, and a leaf-spot of the sugar beet (*Beta vulgaris* L.), and consequently their relationship to each other has not been recognized. The leaf-spotting was attributed by Oudemans[1] to *Phyllosticta betae* and later by Prillieux and Delacroix[2] to *P. tabifica*. Frank[3] believed the latter organism to be identical with his root-rot fungus, *Phoma betae*, but on account of generic differences no combination of names was made. Hedgcock[4] pointed out for the first time a definite connection between Phyllosticta on the leaf and Phoma on the root. Peters[5] and Edson[6] give evidence that the fungus which produces leaf-spotting is also a cause of damping-off. The present investigation shows that the leaf-spot and the root-rot organism are the same and points out that the entire life cycle of the fungus must be considered in any interpretation that is made of the disease phenomena. The name "*Phoma betae* (Oud.) Fr." is deemed by the writers and by Edson[6] to be correct and inclusive; however, the generic name "Phyllosticta" is retained in this paper for the organism isolated from leaves.

SYMPTOMATOLOGY

A mature, normally developed spot of *Phoma betae* on the sugar-beet leaf varies in size from 1 to 2 cm. in diameter and is usually light brown in color. At times such spots show concentric rings of growth, the different zones being outlined by pycnidia. There is no sharp differentiation (Pl. XXVII) between the infected area and the surrounding tissue, owing to the lessened activity of the beet leaf at the time the organism is growing in the leaf tissue. This accounts for the comparatively large size of the spot and its rather diffusive character. The spots which

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[1] Oudemans, C. A. J. A. Aanwinsten voor de flora mycologica van Nederland van Juli 1875 tot Juli. 1876. *In* Nederland. Kruidk. Arch., s. 2, deel 2, stuk 3, p. 181. 1877.

[2] Prillieux, E. E., and Delacroix, Georges. Complément à l'étude de la maladie du cœur de la betterave. *In* Bul. Soc. Mycol. France, t. 7, p. 23-25, pl. 3. 1891.

[3] Frank, A. B. Phoma Betae, ein neuer Rübenpilz. *In* Ztschr. Pflanzenkrank., Bd. 3, p. 90-92. 1893.

[4] Hedgcock, G. G. Proof of the identity of Phoma and Phyllosticta on the sugar beet. *In* Jour. Mycol., v. 10, p. 2-3. 1904.

[5] Peters, Leo. Ueber die Erreger des Wurzelbrandes. *In* Arb. K. Biol. Anst. Land- u. Forstw., Bd. 8, Heft 2, p. 229-239. 1911.

[6] Edson, H. A. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. *In* Jour. Agr. Research, v. 4, no. 2, p. 135-168. 1915.

first appear are generally brown, rarely red, in color. The latter color suggests that the lesion was first produced by some injury which probably caused the formation of carotin, the fungus later gaining an entrance. Typical spots grow rapidly and after 10 days or 2 weeks the black, somewhat erumpent pycnidia develop.

During the growing season of 1912 at Rocky Ford, Colo., observations were made of the different types of Phoma spots that were found to occur. Small brown spots were first noted on old mature leaves in the early part of July. Cultures made from such spots developed colonies of *Cercospora beticola* and *Phoma betae*. Several small red spots collected somewhat later gave either pure cultures of *P. betae* or a mixture of Phoma and an Alternaria. It would seem that the earliest spots contained more than one organism, owing probably to the fact that insect wounds made it easy for various fungi to enter. These spots frequently did not enlarge, showing that the organism had gained no sure foothold. By the last of July or the first part of August large, light-brown, typical spots yielded pure cultures of *P. betae*. Such spots always occurred on those leaves which were old and showed symptoms of yellowing. Consequently on a normal beet plant only a few leaves were infected, but on a plant that was physiologically weakened as a result of rot caused by *Rhizoctonia solani* or of some other factor inimical to plant growth many leaves were found to have typical spots. This observation was confirmed during the season of 1914 at Madison, Wis., where many of the leaves on the "mother beet" plants were found to be infected with Phoma. The roots from which these plants had grown had been more or less affected by various storage rots during the preceding winter, and consequently the vitality of the plants was greatly lowered.

The leaves attacked on the normal and abnormal plants showed the same symptoms of age. Thus, it would seem from field observations that the age of the leaf becomes the important factor in its susceptibility to the disease, and this is upheld in controlled experiments.

AGE AS A FACTOR IN LEAF SUSCEPTIBILITY

Practically all inoculation experiments carried on in 1912 to determine the connection between *Phoma betae* and *Phyllosticta betae* gave negative results. In all the preliminary studies the pycnospores of Phoma from the root and of Phyllosticta from the leaf were suspended in sterile water and either sprayed or smeared on leaves of all ages. Out of 150 inoculations thus made there were only four infections, and these occurred on old, yellow leaves, indicating that the organism is only rarely able to penetrate the unbroken epidermis. Later work has shown that even at the most favorable age the great majority of infections take place through some lesion on the leaf surface.

It was found, after making a large number of counts, that the maturity and the relative age of the different leaves of a beet plant could be determined by taking an average of the number of stomata on a given area at the base, middle, and apex of the leaf.[1] Numerous preliminary determinations showed that within certain ranges the number of stomata that occurred on either surface of the leaf was indicative of its age, so, for convenience, all subsequent counts were made on the upper surface. It was ascertained that leaves with 53 to 100 stomata per square milimeter could be considered as mature and were so designated. Every leaf in the outermost whorls on all plants examined gave stomatal counts within this range. Presumably the cells of such leaves had reached their maximum growth and their greatest metabolic activity. Young mature leaves which had a stomatal count per square milimeter of 92 to 133 were usually taken from a medium position on the plant and were metabolically active, although they had not as yet reached their greatest size. Leaves which had 134 or more stomata per square milimeter were very immature and were located near the heart growth of the plant.

In order to determine which were the most susceptible to infection by *Phoma betae*, 21 needle lesion inoculations on young leaves, 39 on medium-aged leaves, and 90 on old, mature leaves were made. Only 34 infections developed, and these were on the old, mature leaves. A comparable series of inoculations made with *Phyllosticta betae* gave 25 infections from 91 inoculations on old, mature leaves, no infections from 12 inoculations made on medium-aged leaves, and none from 42 made on young leaves. On the petioles 50 inoculations gave no infection with either organism. The number of infections was not increased when the plants were covered with bell jars or pots, but the infected areas appeared somewhat sooner than on uncovered plants. Typical spots developed, if at all, from two to four days after inoculating; however, this incubation time is in all probability lengthened under less favorable field conditions. (See Table I.)

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[1] The stomatoscope originated by Prof. F. E. Lloyd was made available for the work through the kindness of the Alabama Polytechnic Institute. At times an adaptation of the stomatoscope with an ordinary microscope was employed.

TABLE I.—*Results of inoculating sugar-beet leaves of different ages [a] with Phoma betae and Phyllosticta betae*

Age of leaves and series No.	Phoma betae.				Phyllosticta betae.			
	Average number of stomata per sq. mm. of upper leaf surface.	Number of inoculations per leaf.	Condition of leaf four days after inoculation.	Number of infections per leaf.[b]	Average number of stomata per sq. mm. of upper leaf surface.	Number of inoculations per leaf.	Condition of leaf four days after inoculation.	Number of infections per leaf.[b]
Old leaves [c] (series 1).	62.8	3	Yellowing	2	71.0	3	Yellow	3
	73.8	3	Old mature	3	60.1	3	Old mature	3
	60.1	3	Yellowing	3	61.5	3	Old yellow	3
	68.3	3	Beginning to yellow	3	71.0	3	....do	3
	79.2	3	Slightly yellowed	3	87.4	3	Mature	2
	76.5	3	Yellowing	2	82.0	3	Yellowing	3
	61.5	3	Old yellowing	3	90.2	3	Old mature	3
	73.8	3	Yellowing	3	71.0	3	Beginning to yellow	2
	60.1	3	....do	3	90.2	3	Mature	3
	100.0	3	Old mature	2	76.5	3	Old yellow	0
	90.2	3	....do	3	98.4	3	Mature	0
	65.6	3	Beginning to yellow	3	76.5	3	Yellowing	0
	82.0	3	Old mature	1	90.2	3	Old mature	9
	68.3	3	....do	0	87.4	3	....do	0
	85.2	3	Mature	0	71.0	3	Mature	0
	88.1	3	....do	0	60.0	3		0
	82.0	3	....do	0	82.0	3	Mature	0
	98.4	3	....do	0	71.0	3	Old mature	0
	87.4	3	Old mature	0	84.7	3	Mature	0
	84.0	3	....do	0	95.6	3	....do	0
	82.0	3	....do	0	82.0	3	Beginning to yellow	0
	82.0	3	....do	0	82.0	3	Mature	0
	65.6	3	Mature	0	68.3	3	....do	0
	84.7	3	Old mature	0	73.8	3	....do	0
	82.0	3	....do	0	92.9	3	....do	0
	86.9	3	Mature	0	86.1	3	....do	0
	82.0	3	Old mature	0	84.7	3	....do	0
	73.8	3	....do	0	84.7	3	....do	0
	84.0	3	....do	0	98.4	3	....do	0
	95.6	3	....do	0	92.9	3	....do	0
					98.4	3	....do	0
					87.4	3	Beginning to yellow	0
					79.2	3	Mature	0
Total..	90			34		99		25
Old leaves [c] (series 2).	65.6	4	Slightly yellow	1	84.7	4	Yellow	4
	60.1	4	Beginning to yellow and dying.	3	87.4	4	Still green	0
	82.0	4	Yellow	3	79.2	4	Slightly yellow	0
	76.5	4	....do	4	82.0	4	Still green	0
	65.6	4	Yellow and dead	4	82.0	4	....do	0
	79.2	4	Beginning to yellow	1	71.0	4	....do	0
	84.7	4	Quite yellow	4	79.2	4	Rather more green than yellow.	2
	73.8	4	Yellowing	2	76.5	4	Yellowing	4
	57.4	4	....do	4	84.7	4	Yellow and dying	4
	62.8	4	....do	2	73.8	4	Still rather green	1
	87.4	4	Still somewhat green.	4	73.8	4	Yellow	4
	87.4	4	Still green	0	73.8	4	....do	4
Total..	48			32		48		23
Young mature leaves [d] (series 3).	106.6	3		0	101.1	3		0
	103.8	3		0	106.6	3		0
	117.5	3		0	109.3	3		0
	103.8	3		0	110.7	3		0
	110.7	3		0				
	103.8	3		0				
	109.3	3		0				
	110.7	3		0				
	103.8	3		0				
	106.6	3		0				
	112.7	3		0				
	106.6	3		0				
	117.5	3		0				
Total..	39						12	

[a] Inoculations made on plants in the greenhouse of Bureau of Plant Industry, at Washington, D. C., on January 15 and 24, 1914.
[b] *Phoma betae* was reisolated from all infected spots that are indicated in series 1 and 2.
[c] The leaves in this series occupied the outermost position on the beet plants.
[d] The leaves designated as "young mature" were those occupying a medium position in the plant growth.

TABLE I.—*Results of inoculating sugar-beet leaves of different ages with Phoma betae and Phyllosticta betae*—Continued

Age of leaves and series No.	Phoma betae.				Phyllosticta betae.			
	Average number of stomata per sq. mm. of upper leaf surface.	Number of inoculations per leaf.	Condition of leaf four days after inoculation.	Number of infections per leaf.	Average number of stomata per sq. mm. of upper leaf surface.	Number of inoculations per leaf.	Condition of leaf four days after inoculation.	Number of infections per leaf.
Very immature or "heart" leaves ᵃ (series 4).	142.1	3	....	o	133.9	3	....	o
	153.0	3	....	o	174.9	3	....	o
	152.5	3	....	o	169.4	3	....	o
	139.4	3	....	o	143.5	3	....	o
	147.6	3	....	o	164.0	3	....	o
	153.0	3	....	o	169.4	3	....	o
	155.8	3	....	o	136.6	3	....	o
					147.6	3	....	o
					180.4	3	....	o
					144.8	3	....	o
					172.2	3	....	o
					232.3	3	....	o
					158.5	3	....	o
					172.2	3	....	o
	Total ..	21	....	....	....	52	....	....

ᵃ "Heart" leaves occupied the central portion of the plant.

The data recorded in Table I (series 1, 3, and 4) show that the old, mature leaves of the sugar-beet plant or those leaves which are beginning to yellow are the only ones that are susceptible to *Phoma betae*. In order to corroborate this point, additional inoculations were made on leaves that were deemed to be in this condition at the time of inoculating. Out of 48 inoculations with *Phoma betae* 32 typical spots were produced, and with a like number of inoculations with *Phyllosticta betae* 23 infections developed. It will be noted in Table I (series 2) that the inoculations which did not produce infections were made on leaves that were evidently not correctly determined as to their degree of maturity, since, as a rule, they had not yet attained this age at the time the infections should have developed. The cultures in this series were obtained from reisolations of Phoma and Phyllosticta infections of series 1.

The original cultures used for the inoculations with *Phoma betae* were obtained from Mr. H. A. Edson, of the Bureau of Plant Industry, who isolated the organism from rotted sugar beets kept in storage at Longmont, Colo. Those of *Phyllosticta betae* used in the inoculations were obtained from heavily infected sugar-beet leaves that had been collected in Colorado during the growing season of 1913. Puncture inoculations used exclusively in this experiment were made from a suspension of the pycnospores in sterile water.

DISSEMINATION OF PHOMA BETAE

Such agencies as beet balls,[1] wind, irrigation water, insects, and dung play an important part in the distribution of *Phoma betae* in the field.

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[1] Edson, H. A. Op. cit., p. 141.

From 44 plate cultures exposed (2 plates at a time) for 15 to 30 minutes near the open ground surface in various beet fields at different times from June 7 to September 10, 1912, inclusive, at Rocky Ford, Colo., 50 colonies of *P. betae* were obtained. The fungus was present in the air at temperatures which varied from 68° to 115° F. at the ground surface, with relative humidities from 39 to 71 per cent. These readings were taken during the time that the plates were exposed. The organism was not obtained from plates exposed during one night, an experiment which was of necessity limited. Its presence in the air seemed to be dependent on the humidity, a high humidity apparently causing the pycnidia to expel their spores, while a subsequent decrease in the relative humidity caused the spores to escape into the air.

At certain times *P. betae* occurs abundantly in irrigation water. This is particularly true late in August and early in September. The pycnidia are well formed on the leaves by this time, and if moistened they burst and many spores are expelled. Samples of irrigation water which was either standing between the rows or had drained to the lower portions of the beet fields about one day after irrigation yielded Phoma in several cases in the tests made in 1912. Thirty-three colonies of *P. betae* in plate cultures were obtained from 23 c. c. of water representing four such samples, while 3 c. c. of water flowing through a field yielded nine colonies in cultures.

Three species of insects have been found to be carriers of the fungus to only a slight extent. Two culture tests made with the moth of the beet webworm, *Loxostege sticticalis* L., yielded many colonies of *P. betae* in the latter part of July, while cultures made at later intervals gave negative results. Several tests made of the alkali beetle, *Monoxia juncticollis* Say, and the larvæ of the woolly bear (yellow), *Diacrisia virginica* Fab., yielded only a few colonies of the fungus.

Phoma betae may occur in abundance in the dung present in feed yards where beet tops have been fed. It is not to be concluded that the presence of the organism here indicates that it can survive a passage through the alimentary tract of cattle or sheep, but rather that the fungus is viable in dung after the ordinary method of feeding beet tops where they · are not entirely consumed. In one test made early in January, 1913, 36 colonies of *P. betae* were obtained from nine small drops of strong manure decoction.

FACTORS INIMICAL TO VIABILITY OF PHOMA BETAE IN BEET LEAVES

DRY HEAT

The thermal death point of *Phoma betae* in sugar-beet leaf tissue exposed for half an hour to dry heat is between 80° and 90° C. Seventy isolations of *Phoma betae* were made from spots on leaves exposed at 70° for half an hour. At 80° two colonies developed in cultures made from approxi-

mately the same amount of material, and at 90° and 100° none were obtained. It is evident, therefore, that the fungus would be rendered harmless when infected beet tops are dried in a pulp drier.

OVERWINTERING UNDER FIELD CONDITIONS

Phoma betae has been found to be present to a slight extent in the soil of old sugar-beet fields. It was isolated from samples of finely divided soil taken during March and April, 1912, from fields near Rocky Ford, Colo., which had been in sugar beets for several years. Four colonies were obtained from 0.05 gm. of a surface sample, while from cultures made from 0.25 gm. representing two different first-foot samples eight colonies were obtained. No growth of the fungus occurred in cultures made from second- and third-foot samples. Although the tests were continued throughout May, June, July, and the first part of August, no further isolations were made.

About the middle of October, 1912, sugar-beet leaves which were infected with *P. betae* were mixed with soil to the depth of 6 inches in a box and left exposed to outdoor weather changes. No cultures of the organism could be obtained from these leaves after 3 months. However, different results were obtained when the leaves were buried at various depths in the ground. It was found that the fungus was viable at the end of 3 months in leaves which had been buried at depths of 1 to 5 inches or had been kept in the interior of a pile of hayed beet leaves. The organism was isolated from leaves buried at depths of 1 to 5 inches after 5 months, but there was no development from the leaves buried 6 to 8 inches. At the end of 12 months no growth of *P. betae* was obtained at any depth, and the leaves were practically all disintegrated. However, the viability of the fungus was not impaired in dried leaves stored under herbarium conditions for over 2 years.

The maximum temperature of the air from October, 1912, to September, 1913, inclusive, the time of the overwintering experiment, varied from 4° to 102°, the minimum from −20° to 72° F. The maximum temperature of the ground at a depth of 5 inches from December, 1912, to May, 1913, inclusive, varied from 42° to 92°, the minimum from 22° to 51° F. During the 12 months of the experiment there was 9.34 inches of rainfall and snow, mostly during April, May, June, and July. There was no precipitation during November and December of 1912, but there occurred 0.2 inch in January, and 0.4 inch in October, 1913. During this time the lowest soil and air temperatures were registered.

It appears, then, that the results on the viability of the organism obtained from covering the leaves with soil in boxes are not comparable to those obtained under field conditions. The temperature of the air varies from that of the soil to such a degree that accurate results for field comparison can not be obtained in such an experiment. A period of one year seems sufficient to eliminate *Phoma betae* from infected beet-leaf material left in the field, although there is a probability that the

fungus mycelium may remain dormant for a longer period of time in a sugar-beet root or "mother beet" stalk. The writers have found no evidence of a perfect stage of the organism.

The leaves for the outdoor-exposure experiments were buried in such a manner that examination was rendered convenient and accurate. The following method was suggested by Mr. W. A. Orton, Pathologist in Charge of Cotton- and Truck-Disease and Sugar-Plant Investigations, Bureau of Plant Industry. The soil was removed to the depth required and a piece of 2-inch mesh wire was placed on the exposed ground surface. The layer of leaves was then packed over this, another layer of wire added, and then soil to the depth desired. In examining the leaves at any time the layer of wire served to show the position of the leaves, and definite spots could be taken for cultural material. The effect of outdoor winter conditions on the viability of *Phoma betae* in infected beet tops is given in Table II.

TABLE II.—*Effect of different methods of overwintering on the viability of Phoma betae in infected beet leaves*

Method of treatment of infected leaves.	Length of exposure.	Number of cultures made.[a]	Number of isolations.[b]
	Months.		
Buried in soil in box............................	3	3	None.
Do..	4	7	None.
Do..	7	6	None.
Buried 1 inch in ground.........................	6	21	Many.
Do..	8	21	Few.
Do..	10	4	Few.
Do..	11½	8	None.
Buried 2 inches in ground.......................	6	19	8.
Do..	10	5	Few.
Do..	11½	8	None.
Buried 3 inches in ground.......................	6	21	Many.
Do..	10	4	None.
Do..	11½	8	None.
Buried 4 inches in ground.......................	6	19	Many.
Do..	10	6	None.
Do..	11½	8	None.
Buried 5 inches in ground.......................	6	20	Many.
Do..	10	5	None.
Do..	11½	8	None.
Buried 6 inches in ground.......................	6	20	None.
Do..	10	4	None.
Do..	11½	8	None.
Buried 7 inches in ground.......................	6	15	None.
Do..	8	10	None.
Do..	10	4	None.
Do..	11½	8	None.
Buried 8 inches in ground.......................	6	20	None.
Do..	8	10	None.
Do..	10	4	None.
Do..	11½	8	None.
Interior of "hayed" pile of beet tops..............	3½	30	6.
Do..	4	8	1.
Do..	6	6	2.
Do..	11½	8	None.
Left in field on surface of ground.................	5½	11	6.

[a] String-bean agar was used for all cultures.
[b] The number of spots used for each cultural plate varied from 1 to 4 or 5.

ENSILAGE

The process of siloing infected beet tops has been found to be sufficient to kill *Phoma betae*. In the ensilage experiments carried on during the winters of 1912 and 1913 it was ascertained that the organism was viable at the time the silage was made, but could not be isolated after the tops had been ensiled for two months. A medium composed of somewhat diluted silage material was also inimical to the growth of the fungus. Detailed data will be published later in connection with the relation of *Cercospora beticola* to ensiled beet tops.

SUMMARY

A typical spot of *Phoma betae* (Oud.) Fr. is light brown in color, 1 to 2 cm. in diameter, and has scattered over its surface numerous pycnidia, at times concentrically arranged. Such spots on a normal beet plant usually appear during July and August on the old leaves near the ground. If the plant is physiologically low, all except the heart leaves may become infected.

Phoma betae produces a characteristic infection on leaves that have a stomatal count of 60 to 100 per sq. mm. of upper leaf surface.

The pycnospores of the fungus may be disseminated by such agencies as beet balls, wind, irrigation water, insects, and dung.

The thermal death point of *Phoma betae* in the leaf tissue exposed for one-half hour to dry heat is between 80° and 90° C. The fungus is dead in infected leaves after three months' storage in soil in boxes exposed to outdoor conditions, while its life becomes extinct in leaves buried in the ground only after five to eight months, depending on the depth of cover. The fungus can not survive the process of ensiling the beet tops.

PLATE XXVII

NOTES ON THE HYDROCYANIC-ACID CONTENT OF SORGHUM

By J. J. WILLAMAN and R. M. WEST, *Assistant Chemists, Agricultural Experiment Station of the University of Minnesota*

INTRODUCTION

In the course of our work on the chemistry of *Sorghum vulgare* it was thought desirable to study its content of hydrocyanic acid (HCN) under Minnesota conditions. Many instances are on record of the poisoning of cattle from feeding on growing sorghum cane, and some of these cases have been definitely proved to be due to hydrocyanic acid, which occurs in sorghum as a constituent of the glucosid dhurrin (6).[1]

The factors which affect the amount of this glucosid in the plant have received some attention. All investigators have found that it decreases as the plant matures. Maxwell (8) states that sorghum is not fed with safety until after the seeds begin to develop; Brünnich (4), that it should not be fed until the seeds are fully matured. Avery (2) says that the amount of hydrocyanic acid is greater in stunted plants, while Alway and Trumbull (1) found that yellow, stunted plants contained less of the acid than the green, vigorous plants in the same field. Maxwell (8) believes that the amount of the glucosid is dependent on the character of the soil, soils rich in nitrogen producing plants richer in the glucosid. Brünnich (4), in experiments with sodium nitrate in Queensland, found that the fertilized plants contained slightly more hydrocyanic acid than those unfertilized and concluded that heavy nitrogenous soils and favorable climatic conditions increase the amount of the acid. His findings were corroborated by Alway and Trumbull (1). Brünnich (5) also found that millet (*Panicum miliaceum*) behaved similarly to sorghum. Schröder and Dammann (10), in Uruguay, report an increase in prussic acid due to the use of sodium nitrate as a fertilizer. Balfour (3) noticed that plants infected with *Aphis sorghi* contained more hydrocyanic acid than uninfected plants. These are the main facts which have been published in the literature concerning the occurrence of a cyanogenetic glucosid in sorghum.

Samples of the 1913 crop of cane grown on the farm of the University of Minnesota were analyzed about the middle of August, and the acid was found to be absent in all cases. As it has often been proved to persist in the plant to a later period, this result was considered unusual, and it was decided to repeat the work the next season. On analyzing a sample of plants 6 inches high, taken on June 26, 1914, hydrocyanic

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[1] Reference is made by number to "Literature cited," p. 185.

acid to the amount of 0.058 per cent of the dry matter was found. In view of the inconsistencies found in the above reports and in our analyses, it was deemed advisable to study the question further, especially with respect to the production and distribution of the prussic acid in sorghum.

EXPERIMENTAL WORK

Two of the plots of sorghum grown on the university farm in rich, fertile soil were selected, and the second row of each was treated with dried blood at the rate of 800 pounds per acre. This left five check
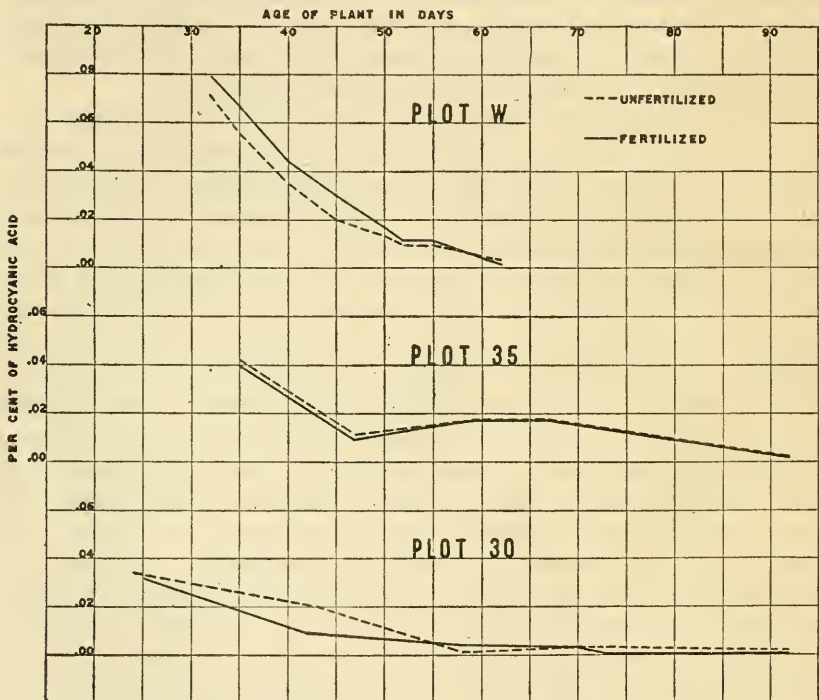
FIG. 1.—Curve showing the effect of available nitrogen on the hydrocyanic-acid content of sorghum. The percentage of hydrocyanic acid is based on dry matter.

rows unfertilized. The fertilizer was applied on July 2, when the plants were about 8 inches high. Samples of the crop were taken at intervals thereafter and analyzed for hydrocyanic acid. At the same time six rows of sorghum (Early Rose variety) were planted in some very poor, sandy soil. The first row was left as a check, the second treated with dried blood at the rate of 100 pounds per acre, the third with 200 pounds, the fourth left as a check, the fifth with 400 pounds, and the sixth with 800 pounds per acre.

The six rows planted on the sandy plot of ground thrived very poorly. They were slow in sprouting, owing to dry weather, and for the first few weeks grew very slowly. The soil was too poor to support plant growth

adequately, and after about eight weeks of slow growth the leaves turned yellow and frosts came before the plants were well headed out. This condition, together with the use of the fertilizer, gave us an opportunity to study the occurrence of prussic acid in poorly nourished plants and to compare them with those having a better supply of nitrogen.

In the samples from this plot the whole plant was ground up for the determination of hydrocyanic acid. In general, the cane in the rows which received fertilizer grew a little better than that which did not, but Table I shows that the increase in hydrocyanic acid is inappreciable. It can be detected only by comparing the average of the two check rows with the average of Rows V and VI, which received the heaviest applications of fertilizer. This comparison is shown in figure 1, plot W, the dotted lines representing the average of Rows I and IV and the solid line that of Rows V and VI. In a measure these results substantiate the work of some of the investigators mentioned above, in that the soils with the better supply of nitrogen were found to have produced plants with a higher content of hydrocyanic acid. The difference, however, is very slight, and the findings of Alway and Trumbull (1) rather than those of Avery (2) are supported, for the reason that the stunted plants showed less hydrocyanic acid than the thrifty ones. In these plots the amount of the acid in the early stages was higher than in the plots having good soil (fig. 1, plots 30 and 35), but it persisted through a much shorter period of the plant's life.

TABLE I.—*Effect of available nitrogen on the hydrocyanic-acid content of sorghum*

[Percentage of hydrocyanic acid is reported on a dry-matter basis]

PLOT W

Row or plot and sample number.	Height.	Age.	Percentage of hydrocyanic acid.		
			Stalks.	Leaves.	Whole plant.
Row I:[a]	*Inches.*	*Days.*			
1	18	31			0. 083
2	28	38			. 032
3	39	52			. 000
4	42	55			. 007
5	61	62			. 002
Row II:[b]					
6	19	31			. 083
7	31	38			. 027
8	40	45			. 025
9	57	62			. 000
Row III:[c]					
10	20	31			. 083
11	34	38			. 022
12	40	45			. 032
13	56	62			. 005
Row IV:[a]					
14	26	32			. 064
15	34	39			. 043
16	40	45			. 024
17	56	62			. 007

[a] Check.   [b] Fertilized at rate of 100 pounds per acre.   [c] Fertilized at rate of 200 pounds per acre.

TABLE I.—*Effect of available nitrogen on the hydrocyanic-acid content of sorghum*—Con.

PLOT W—continued

Row or plot and sample number.	Height.	Age.	Percentage of hydrocyanic acid.		
			Stalks.	Leaves.	Whole plant.
Row V:[a]	*Inches.*	*Days.*			
18	26	32			0. 068
19	38	39			. 045
20	42	52			. 021
21	59	62			. 000
Row VI:[b]					
22	21	32			. 095
23	32	39			. 049
24	43	52			. 000
25	40	55			. 007
26	58	62			. 003

PLOTS 35 AND 30

Row or plot and sample number.	Height.	Age.	Stalks.	Leaves.	Whole plant.
Plot 35 (feterita):[c]					
27	22	24	0. 077	0. 025	
28	39	35	. 053	. 032	0. 041
29	57	47	. 010	. 013	. 011
30	77	59	. 0068	. 037	. 017
31	90	67	. 0009	. 033	. 017
32	83	92	. 0000	. 008	. 0021
Plot 35 (feterita):[b]					
33	22	24	. 077	. 052	
34	36	35	. 065	. 027	. 040
35	60	47	. 0047	. 015	. 009
36	81	59	. 0068	. 037	. 017
37	89	67	. 0068	. 034	. 017
38	85	92	. 0000	. 018	. 0035
Plot 30 (Orange sorgo):[c]					
39	29	25	. 050	. 019	. 033
40	59	43	. 000	. 042	. 020
41	102	58	. 000	. 005	. 0011
42	103	70	. 000	. 022	. 0034
43	100	73	. 000	. 021	. 0022
44	96	92	. 000	. 0065	. 0013
Plot 30 (Orange sorgo):[b]					
45	28	25	. 050	. 017	. 032
46	60	42	. 000	. 019	. 0093
47	96	58	. 000	. 014	. 0038
48	105	70	. 000	. 016	. 0027
49	95	73	. 000	. 0036	. 0005
50	97	92	.‚000	. 0046	. 0007

[a] Fertilized at rate of 400 pounds per acre.   [b] Fertilized at rate of 800 pounds per acre.   [c] Check.

In Table I are also brought together the analyses of the samples from the plots on the fertile ground on the university farm. Plot 35 was feterita, a variety of sorghum; plot 30 was Orange sorgo. In these experiments the leaves were stripped from the stalks, the hydrocyanic acid determined on each portion, and the percentage of the acid in the whole plant calculated on the basis of the relative proportion of dry matter in the leaves and stalks. In figure 1 the results for the whole plant are

shown graphically.   Here, again, there appears only a slight difference
in the hydrocyanic-acid content due to the added nitrogen fertilizer; but
contrary to the effect shown in Table I and the curve for plot W, the
slightly lower percentage of prussic acid was found in the plants from
the fertilized row.   In plot 35 the decrease is insignificant, and in plot 30
the two curves cross each other twice.   Taking into consideration the
results from all three plots, it appears that on soils deficient in nitrogen
added nitrogen will slightly increase the prussic acid in sorghum; but
that with a plentiful supply of nutrients in the soil added nitrogen does
not affect the amount of the acid in the plants.

A plentiful supply of nitrogen in the soil will permit the maintenance
of a definite amount of prussic acid at a given stage of growth; but it may
be that this amount is not absolutely required and that if the supply of
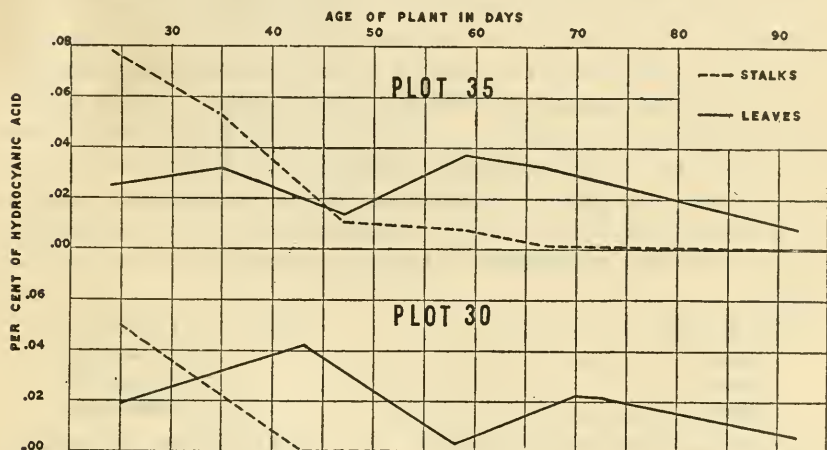
Fig. 2.—Curve showing the distribution of hydrocyanic acid in sorghum. The percentage of hydro-
cyanic acid is based on dry matter.

nitrogen is deficient the plant maintains the equilibrium of other nitrog-
enous compounds at the expense of the prussic acid.

Figure 2 shows the distribution of the prussic acid in the two varieties
of sorghum, and an interesting varietal difference appears.   The stalks
of feterita contain in the early stages a relatively high percentage of
prussic acid, and the acid persists in small amount through most of the
life of the plant.   The stalks of Orange sorgo, however, show less of the
acid in the beginning, and it disappears entirely by the fortieth day.
Both plots show an increase of the acid in the leaves during the early
stages, and later a decrease; the acid in the leaves had not completely
disappeared in this experiment by the ninety-second day.   Evidently
the cyanogenetic glucosid is related to the vital processes of the plant, as
it occurs in the largest quantity in those parts of the plant which are
most active photosynthetically and during those stages when the plant

is developing most rapidly. Treub (12) and Ravenna and Zamorani (9) are of the opinion that hydrocyanic acid is a necessary intermediate product in the formation of proteins. As such, the quantity present at any one time might be subject to such variation as this experiment shows.

Further experiments will be carried on next season (1915) to determine, if possible, just what effect variety and climatic conditions may have on the prussic-acid content of sorghum, as well as the function of the acid in the metabolism of the plant.

METHOD OF DETERMINING HYDROCYANIC ACID

For the determination of hydrocyanic acid the colorimetric method of Francis and Connell (7) was used, with one important modification. It was found that when the macerated tissue was distilled with sulphuric acid according to their method the distillate became yellow, and when subsequently treated with ferric chlorid a greenish or brownish precipitate was formed which masked the color of the thiocyanate. Enzym hydrolysis was therefore resorted to. Slade (11) digested the ground tissue for 12 hours at room temperature, making use of the enzym which is always found in a plant in conjunction with a cyanogenetic glucosid. But we found that at 40° to 45° C. complete hydrolysis was obtained in two hours or less, as portions of the same sample gave the following results:

Time of digestion.	HCN in 10 gm. of ground material.
2 hours	0. 00040 gm.
4 hours	0. 00040 gm.
6 hours	0. 00025 gm.

In all our work 2-hour digestions were used, and the hydrocyanic acid distilled and determined in the usual way.

CONCLUSIONS

The following points may be presented as a summary of these notes:

(1) When sorghum is grown on poor, infertile soil, added nitrogen may slightly increase the amount of hydrocyanic acid in the plant. With a fertile soil and abundant nitrogen this effect may not be produced.

(2) During the first three or four weeks of the plant's life the prussic acid is concentrated in the stalks. Then it rapidly decreases and disappears there, but apparently persists in the leaves in decreasing percentages until maturity.

(3) Climate and variety may be more important factors than soil nitrogen in determining the amount of the acid in the plant.

(4) Complete hydrolysis of the glucosid is obtained by digesting the macerated tissue for two hours at 40° to 45° C.

LITERATURE CITED

(1) ALWAY, F. J., and TRUMBULL, R. S.
    1910. On the occurrence of prussic acid in sorghum and maize. *In* Nebr. Agr. Exp. Sta. 23d Ann. Rpt., 1909, p. 35–36.

(2) AVERY, Samuel.
    1902. Laboratory notes on poison in sorghum. *In* Jour. Comp. Med. and Vet. Arch., v. 23, no. 11, p. 704–706.

(3) BALFOUR, Andrew.
    1904. Cyanogenesis in Sorghum vulgare. *In* 1st Rpt. Wellcome Research Lab. Gordon Mem. Col. Khartoum, [1903/04], p. 46–48.

(4) BRÜNNICH, J. C.
    1903. Hydrocyanic acid in fodder-plants. *In* Jour. Chem. Soc. [London], v. 83, pt. 2, p. 788–796.

(5) ——
    1904. Report of the chemistry division. *In* Queensland Dept. Agr. Rpt., 1903/[04], p. 72–78.

(6) DUNSTAN, W. R., and HENRY, T. A.
    1902. Cyanogenesis in plants. II. The great millet, Sorghum vulgare. *In* Phil. Trans. Roy. Soc. London, s. A, v. 199, p. 399–410.

(7) FRANCIS, C. K., and CONNELL, W. B.
    1913. A colorimetric method for determining hydrocyanic acid in plants, with special reference to kafir corn. *In* Jour. Amer. Chem. Soc., v. 35, no. 10, p. 1624–1628.

(8) MAXWELL, W.
    1903. Sorghum poisoning. *In* Queensland Agr. Jour., v. 13, no. 5, p. 473–474.

(9) RAVENNA, Ciro, and ZAMORANI, Mario.
    1909. Nuove ricerche sulla funzione fisiologica dell' acido cianidrico nel Sorghum vulgare. *In* Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat., v. 18, sem. 2, fasc. 8, p. 283–287.

(10) SCHRÖDER, Johannes, and DAMMANN, Hans.
    1911. Zur Kenntnis der aus verschiedenen Hirsearten entwickelten Blausäuremengen. *In* Chem. Ztg., Jahrg. 35, no. 155, p. 1436–1437.

(11) SLADE, H. B.
    1903. Prussic acid in sorghum. *In* Jour. Amer. Chem. Soc., v. 25, no. 1, p. 55–59.

(12) TREUB, Melchior.
    1909. Nouvelles recherches sur le rôle de l'acide cyanhydrique dans les plantes vertes. III. *In* Ann. Jard. Bot. Buitenzorg, v. 23 (s. 2, v. 8), pt. 1, p. 85–118, pl. 18–23.

EFFECT ON SOIL MOISTURE OF CHANGES IN THE SURFACE TENSION OF THE SOIL SOLUTION BROUGHT ABOUT BY THE ADDITION OF SOLUBLE SALTS

[A PRELIMINARY REPORT]

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While temporary research assistant in soils in the Michigan Experiment Station the writer had occasion to do laboratory work in which information was sought as to how far the effect of fertilizer or soil-amendment materials in altering the moisture condition of soil is dependent upon changes produced in the surface tension of the soil solution.

The effect of the materials on the surface tension when in dilute water solutions was taken as an indication of their effect on the surface tension of the soil solution. The surface tension of the dilute solutions, together with their viscosity, specific gravity, and resistance in ohms, is shown in Table I. For comparison like expressions for pure water and for two soil percolates are also introduced.

TABLE I.—*Effect of salts on certain physical properties of the solutions*

Solution.	Specific gravity.	Resistance.	Surface tension, 25° C.	Specific viscosity.
		Ohms.	Dynes.	
Water.................................	1. 0000	[a]180, 000. 0	72. 00	1. 0000
NaNO₃................................	1. 0066	87. 8	72. 33	1. 0049
(NH₄)₂SO₄..........................	1. 0059	65. 5	72. 15	1. 0163
Manure extract......................	1. 0059	89. 5	59. 13	1. 0992
NaCl................................	1. 0070	57. 6	72. 36	1. 0200
KCl.................................	1. 0063	59. 2	72. 24	1. 0011
K₂SO₄...............................	.1. 0080	82. 0	72. 29	1. 0132
CaH₄(PO₄)₂..........................	1. 0060	222. 3	72. 36	1. 0207
Na₂CO₃..............................	1. 0099	71. 8	72. 15	1. 0464
Sandy-loam percolate.................	1. 00257	224. 0	70. 75	1. 0595
Clay-loam percolate..................	1. 00293	190. 0	71. 18	1. 0677

[a] Approximately.

The solutions of the single salts were made by dissolving 10 gm. of Kalbaum's chemically pure salts in 1,000 c. c. of pure water. The changes brought about here in the physical properties of the solutions very probably were greater than those which result in the soil solution from average field application of the salts. The manure extract was obtained by forcing out the liquid contents of approximately equal parts of fresh solid and liquid horse manure and then diluting to about

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the resistance of the sodium-nitrate solution. The soil percolates were obtained by taking the first small portions of solutions percolated through 500 gm. of previously air-dried soil in a percolator tube. The first 15 c. c. were used from sandy-loam soil and the first 20 c. c. from the clay-loam soil. These portions were the densest percolates it was possible to obtain.

All the single salts increased the surface tension to some extent, but in no case was this action marked.[1] The viscosity was also increased in all cases. Changes in the viscosity of the soil solution, while theoretically not affecting the final distribution of moisture reached, should, never-theless, alter the time required for this state to be gained. The small increases in surface tension noted above could hardly be expected to exert any appreciable effect on the moisture content of, or the moisture move-ment in, soil. In this connection the work of Whitney[2] in determining the effect of soluble salts on the surface tension of solutions, which has been used to show that fertilizer salts may exert a significant effect on the soil-moisture content or movement through changes in the surface tension, seemingly has been allowed more weight than it merits. The solutions used by Whitney were either very dense or else saturated, and as such gave changes in the surface tension that would not be com-parable with those arising from the field application of fertilizer salts. The surface tension of the manure extract was much lower than that of water. It is interesting to note that the surface tension of the soil percolates was but little lower than that of water.

Information as to the effect of the materials on the soil-moisture con-tent and movement was secured through the following experimental work. Samples of the sandy-loam and the clay-loam soils from which samples for the soil percolates were obtained were weighed out. The sandy-loam samples weighed 660 gm. each, and the clay-loam samples, 580 gm. each. Fifty c. c. of the dilute water solutions above described, omitting the two soil percolates, were added to each sample. This is at the rate of 1,000 pounds per acre of surface, with the exception of the manure extract. The treated samples were air-dried, mixed, and put into glass tubes 6 cm. in diameter and 20.32 cm. in height. In each case the samples were packed into a volume of 505 c. c., making a soil column approxi-

[1] Values for the surface tension and also for the viscosity of these salts in some cases of densities not far removed from that employed here have been determined by a number of investigators in pure physico-chemical lines. (See Castell-Evans, John. Physico-Chemical Tables for the Use of Analysts, Physicists, Chemical Manufacturers, and Scientific Chemists . . . v. 2, p. 756. London, 1911.) The results given here are not presented as affording any essentially new information along this line. The work was done largely for the purpose of establishing the accuracy of the work to follow on soil percolates and manure extract solutions.

The drop method was used in the surface-tension work, employing the dropping pipette (stalagmometer) of Traube. (See Abderhalden, Emil. Handbuch der biochemischen Arbeitsmethoden. Bd. 5, T. 2, p. 1358. Berlin, Wien, 1912.)

[2] Whitney, Milton. Some physical properties of soils in their relation to moisture and crop distribution. U. S. Dept. Agr. Weather Bur. Bul. 4, 90 p. 1892.

mately 18 cm. in height. The columns were saturated with water, sealed at the tops, weighed, and placed on an air-dried sandy-loam soil. From time to time they were removed, weighed, the moisture content thus determined, and replaced. There was no covering over the bottoms of the tubes, so that the soil columns were in direct capillary contact with the dry soil underneath. The amount of moisture in this undersoil varied from 1 to 3 per cent throughout the experiment. The treatments were run in quadruplicate with each soil. In addition to the treatments already mentioned, there were four check or no-treatment columns with
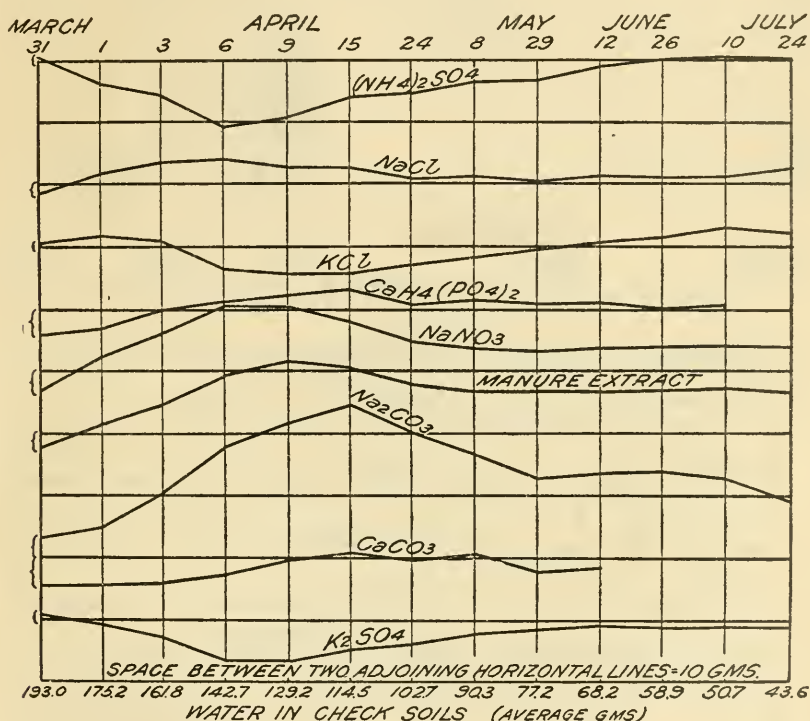
Fig. 1.—Curve showing the differences in the moisture content of treated and check sandy-loam soils.

each soil, and likewise four columns to which 2 gm. of calcium carbonate, at the rate of 4,000 pounds per acre surface, had been added.

The differences in the moisture content throughout the experiment between the treated soils and the check soils are shown in the accompanying curves (fig. 1 and 2). Here the amount of water in the check soils is represented by a horizontal line and the increase or decrease of water in the treated soils over or under this by distance above or below these lines.

The effect of the treatments on the moisture content or water-retaining power of the soils is summarized in Table II.

TABLE II.—*Effect of various fertilizer salts on the moisture content of soils*

Treatment.	Water-retaining power.		
	Increased with—	Not affected with—	Decreased with—
Na_2CO_3	{ Sandy loam.... { Clay loam.....	....	
Manure extract...............	{ Sandy loam.... { Clay loam.....	....	
$NaNO_3$	{ Sandy loam.... { Clay loam.....	....	
NaCl	{ Sandy loam.... { Clay loam.....	....	
$CaCO_3$	....	{ Sandy loam.... { Clay loam.....	
$CaH_1(PO_4)_2$	....	{ Sandy loam.... { Clay loam.....	
KCl	....	Clay loam.....	Sandy loam.
K_2SO_4	....	Clay loam.....	Sandy loam.
$(NH_4)_2SO_4$	....	Clay loam.....	Sandy loam.
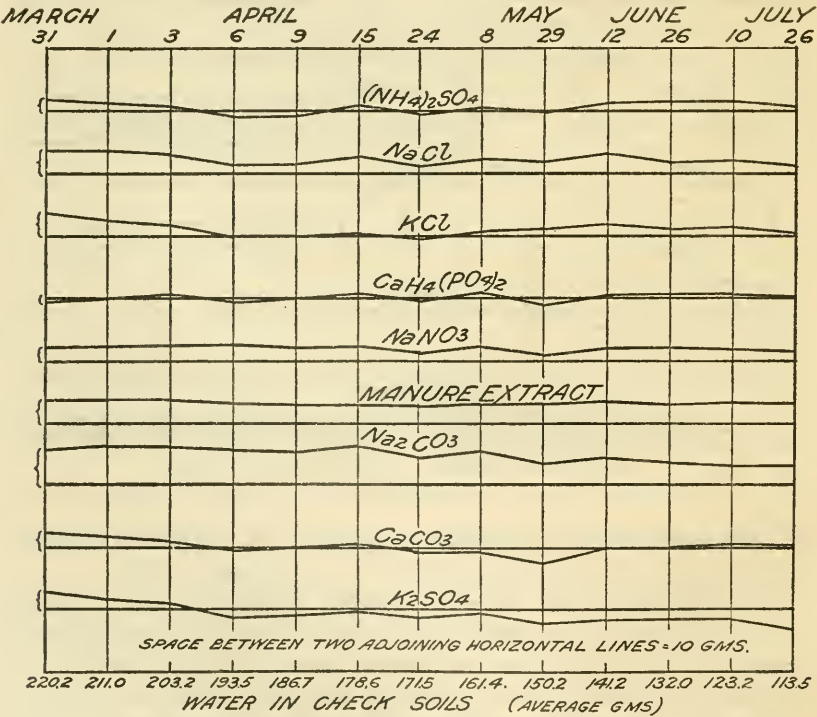
FIG. 2.—Curve showing the differences in the moisture content of treated and check clay-loam soils.

It has already been said that the effect of the single salts on the surface tension of the solutions was too small for any measurable changes in the moisture condition of soil to be expected from this source. In the case

of the manure-extract solution, however, there was a marked decrease in the surface tension, but this change evidently exerted no apparent effect upon the moisture content of the soils treated with the manure-extract solution, for these soils showed an increase in the moisture movement rather than a decrease, which would have resulted from any noticeable action of a decreased surface tension.

The fact that the treatments which influenced to any extent soil-moisture content or movement are those known to have a marked influence on the physical structure of soils points very strongly to the conclusion that herein lies the effect of the salts in this regard. Some information as to the effect of the treatments on the physical structure of the soils in this experiment was obtained by the following work. At the close of the moisture work the soil columns were removed from the tubes and allowed to air-dry. Those found to be intact were selected and equal lengths of the columns, 2½ inches, were removed from their lower ends. The resistance of these sections to a crushing force was determined. The results are shown in Table III.

TABLE III.—*Resistance of sections of the soil columns to a crushing force*

SANDY LOAM

Treatment.	Resistance.	Average resistance.	Treatment.	Resistance.	Average resistance.
	Kilos.	*Kilos.*		*Kilos.*	*Kilos.*
Na₂CO₃............	19.91	19.91	K₂SO₄............	12.68 12.98	12.83
NaNO₃............	16.48	16.48			
Manure extract....	16.36 16.12	16.24	KCl	13.06 12.01	12.54
NaCl	14.25 16.31	15.28			
CaCO₃............	14.87 15.37 12.47	14.24	CaH₄(PO₄)₂......	11.97 12.51 11.39	11.96

CLAY LOAM

Treatment.	Resistance.	Average resistance.	Treatment.	Resistance.	Average resistance.
Na₂CO₃............	92.78 90.08	91.43	CaHPO₄	73.27 75.81 73.27 77.24	74.90
NaNO₃............	88.81	88.81			
NaCl	86.83 82.80 87.07 86.83	85.88	CaCO₃............ KCl	68.68 67.88	68.68 67.88
Check............	75.73 85.01	80.37	K₂SO₄............	67.00 67.09 68.60	67.56
(NH₄)₂SO₄........	79.78 74.08 73.75	75.87			

A comparison of the order of treatments here with that in Table II, in which the effect of the treatments on moisture content or movement is given, shows close correlation between the two. Treatments which

detrimentally affected the structure of the soil and made the soil more close evidently retarded the moisture movement and increased the moisture content, while treatments which promoted the soil structure and made the soil more open hastened the moisture movement and decreased the moisture content.

The results from the work presented in this paper indicate that changes in the surface tension of the soil solution arising from application of fertilizer salts are of no importance in affecting the moisture condition of the soil.

RELATION BETWEEN PUCCINIA GRAMINIS AND PLANTS HIGHLY RESISTANT TO ITS ATTACK

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INTRODUCTION

The intimate relations between host plants and uredine parasites were first carefully investigated by H. Marshall Ward. Ward (10, 12)[1] showed that the relation between the brown-rust of bromes (*Puccinia dispersa* Erikss.) and its host plants might be quite variable. When normal infection took place, as Ward pointed out, a very fine adjustment was made between host and parasite, resulting in a vigorous development of the fungus without immediate serious injury to the host. Indeed, the host seemed sometimes not only not to be injured for a long time but even to be somewhat stimulated. However, in some cases the fungus was found to kill some of the host cells very soon after gaining entrance, and the fungus itself grew but little. A wide range of possibilities was found in varying degrees of infection, establishing the general principle that the success of infection depended largely on the closeness of symbiotic relations set up between the host and the parasite.

Gibson (4) showed that the germ tube of a rust fungus might enter practically any plant, but that after entrance it was unable to produce haustoria and consequently could not live. She found that when varieties of chrysanthemum resistant to *Uredo chrysanthemi* Roze were inoculated with spores of this rust the host cells near the hyphæ were killed, the further growth of the hyphæ being thereby inhibited. Marryat (5) found a similar condition existing between *P. glumarum* Er. and Henn. and host plants partially immune to its attack. The writer (8) has shown that various strains or biologic forms of *P. graminis* Pers. may kill shortly after inoculation comparatively large areas of tissues in host plants that exhibit a considerable degree of resistance to the fungus. Unquestionably the host plant in such cases is often hypersensitive to the fungus, since the fungus kills very early much of

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[1] Reference is made by number to "Literature cited," p. 198-199.

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the host tissue and fails to develop normally, in sharp contrast to the conditions in normal infection. Where extreme incompatibility exists between host and parasite, there is often no externally visible evidence that the fungus has even gained entrance. In fact, it is hardly accurate to speak of the inoculated plant as the host, since the fungus is unable to attain any considerable degree of development in it (7). The work reported in this paper was with such forms and was undertaken in order to determine whether the phenomenon was one of real resistance or an extreme case of hypersensitiveness. Hypersensitiveness is used here to indicate the abnormally rapid death of the host plant cells when attacked by rust hyphæ. It is used in this sense without any implication as to the exact physiological nature of the phenomenon, referring, therefore, only to the facts substantiated by visual evidence.

FORMS OF THE FUNGUS INVESTIGATED

The forms used for study were the following: Oats (*Avena sativa*) inoculated with *Puccinia graminis tritici;* oats inoculated with *P. graminis hordei;* rye (*Secale cereale*) inoculated with *P. graminis* from *Dactylis glomerata* after three generations on oats; wheat (*Triticum* spp.) inoculated with *P. graminis* from *Dactylis glomerata* after three generations on oats; wheat inoculated with *P. graminis avenae;* and barley (*Hordeum* spp.) inoculated with *P. graminis* from *Dactylis glomerata* after three generations on oats. The rusts used, except that from *Dactylis glomerata*, had been confined to the particular host from which they were taken for at least 20 "generations"—i. e., 20 successive transfers had been made in the greenhouse. The rust from *Dactylis glomerata* was taken from rusted plants in the field, inoculated on oats, and then transferred to oats two successive times.

The forms selected represent extreme cases of incompatibility, as is shown by the following table:

Results of inoculations with strains of Puccinia graminis

Form of rust used.	Plant inoculated.	Number of plants inoculated.	Number of plants infected.
Puccinia graminis tritici	Oats	115	0
Puccinia graminis hordei	...do	183	3
Puccinia graminis from *Dactylis glomerata*	Rye	114	0
Do	Wheat	86	0
Do	Barley	58	0
Puccinia graminis avenae	Wheat	283	4

In the case of the rust from *Dactylis glomerata*, some of the inoculations were made directly from the grass and some after one to three generations on oats. They are all grouped in the table, since the rust apparently was not changed after having grown for some time on oats.

EXPERIMENTAL METHODS

The plants used for inoculation were grown in 4-inch pots in the green-house. When they were 6 to 8 days old they were inoculated with an ordinary flat inoculating needle, special care being taken not to injure the tissues in any way. After inoculation the pots were placed in pans containing a little water and then covered with bell jars for 24 or 48 hours, or, in a few cases, longer. The inoculated portions were placed in killing fluid at periods ranging from 48 hours to 12 days after inoculation. Flemming's weaker solution, medium chromo-acetic acid, and picro-acetic acid were used at various times. The material was embedded in paraffin in the usual manner and was cut from 6 to 11 μ thick and then stained. For staining, Harper's modification of the triple stain and Gram's stain, with a counter stain of eosin, gave the best results.

HISTOLOGICAL DETAILS OF HYPHAL INVASION

It is probably superfluous to call attention to the fact that when normal infection, such as occurs, for example, when oats are inoculated with *P. graminis* from *Dactylis glomerata*, takes place, the host cells remain at least apparently normal for a considerable length of time. In the early stages of infection, although the leaf tissues in the infected area may become yellowish in color, it is clearly evident to the naked eye that no extensive and rapid killing of tissues is taking place. When such areas are carefully examined in section, the host cells very frequently appear entirely normal. (Pl. XXVIII, fig. 1.) The hyphæ may grow very vigorously, send haustoria into the cells, and branch profusely without destroying the chloroplasts or in other ways injuring the cell. Even after pustule formation has begun, many of the cells just at the edge of the pustule, where the hyphæ are massed in great numbers, still retain their chloroplasts and are apparently normal in other respects (Pl. XXVIII, fig. 2). It not infrequently happens that cells just under a uredinial sorus, even when the fungus has sent numerous haustoria into them, still retain a number of chloroplasts and seem to have suffered no serious injury. Of course, dead cells are found in a heavily infected region, but in no case does the fungus seem to kill quickly and sharply the cells with which it comes in contact. Normal infection has been described and illustrated quite completely by Ward (9), Evans (2), and the writer (8).

When examination is made, however, of the tissues of a plant that has been inoculated with a rust form which does not grow in it normally, very sharp differences are observable within 48 hours after inoculation. Even in the very early stages it is very evident that normal infection is not taking place and that there is a comparatively violent action and reaction between the plant and the parasite.

The sequence of events in resistant or immune forms is very nearly the same in the different forms studied. The germ tubes form appressoria

over the stomatal slits in a perfectly normal manner, send a protoplasmic process through the slit, and then form the substomatal vesicle. The stimulus to entrance may be negative phototropism, since Fromme (3) has found such a response on the part of uredine germ tubes. Forty-eight hours after inoculation infection threads have frequently already grown into the intercellular spaces and have branched quite profusely. The hyphæ are frequently large and very vigorous in appearance. Haustoria are sometimes sent into the cells of the host; in many cases they are large in size and of normal appearance.

Within a short time after the hyphæ become closely appressed to the host-plant cells, there are usually unmistakable evidences of some deleterious influence upon the host cells. The chloroplasts very often seem to be affected first. They may appear slightly corroded at first and somewhat irregular in outline. They may retain their identity for some time, but more often seem to be clumped together in more or less irregular masses. This appearance may frequently be due to a shrinking of the protoplast from the cell wall, especially when the chloroplasts are quite numerous (Pl. XXVIII, fig. 4 and 5). As the process of disintegration progresses, the outlines of the individual plastids become increasingly fainter until they are scarcely distinguishable. Sometimes, however, the outlines are still visible, although there appears only a more or less uniformly staining mass (Pl. XXVIII, fig. 4 and 5). Eventually the outlines of the plastids become obliterated almost entirely, leaving only a fairly homogeneous, uniformly staining, nongranular mass, with little remaining semblance of structure. In such dead cells, however, the very faint outlines of what were probably chloroplasts may be discernible in some part of the cell (Pl. XXVIII, fig. 6). Sometimes the contents of dead cells appear more or less finely granular, the granules being variously disposed. They may occur in irregular clumps, in beadlike chains, or in various other combinations. More frequently the contents are very nearly homogeneous, with only a few scattering granules.

The action does not always depend upon actual contact. It seems sometimes to precede actual fungous invasion, although in no observed case did it occur very extensively far in advance of actual hyphal invasion. The hyphæ often are very closely appressed to the cell wall, and in such cases the action goes on very rapidly. Quite frequently, however, the chloroplasts on one side of the cell will have been destroyed almost completely, while on the other side, away from the hyphæ, they still appear quite normal. Naturally, of course, all sorts of gradations can be found. The ultimate result is, however, usually the same. The chloroplasts disappear, the nucleus shows definite signs of disintegration, the protoplast collapses, and the cell stands out sharply from the normal ones near it.

Variations occur also in the matter of cells attacked. It seems that sometimes the hyphæ grow over or past cells which apparently escape

injury, and kill those at some distance from the original point of infection. In a few cases it was observed that hyphæ grew from the upper to the lower epidermis, killing a few cells about halfway between the two and killing a number of them near the lower epidermis. Such cases are rather exceptional; the death of the host cells usually follows promptly after the hyphæ reach the cells. Whereas in the case of normal infection pustules with very abundant spore production are being formed within about 7 to 12 days from the time of inoculation, in such cases as those described above a few host cells have been killed and the fungus has reached its limit of development within the same length of time.

The hyphæ do not grow much after the death of the cells. In some cases they were found to be surrounded by dead cells as early as 3½ days after inoculation, and they themselves showed distinct signs of extreme unthriftiness—viz, large vacuoles alternating with coarsely granular areas. Other hyphæ appeared very much as do the older portions beneath an old pustule in cases of normal infection. These hyphæ had grown across the substomatal space and had killed all the mesophyll cells bordering on it, but had not completely killed any deeper lying cells, although some of those just beyond the border cells were somewhat affected. Under such circumstances it is conceivable that the fungus may have died from lack of nourishment, since practically all the food material stored in the spore had probably been used up in the growth of the germ tube along the length of about 10 epidermal cells, in the formation of the substomatal vesicle, and in the growth of the numerous infection threads across the substomatal space. It seems quite possible that the fungus, having exhausted the supply of nutrients stored in the spore, precluded the possibility of its further growth by killing very quickly the first cells with which it came in contact, thus shutting off its only source of food material.

The action is not always as rapid and sharp as in those cases just described. Hyphæ at the end of five days from the time of inoculation have sometimes killed most of the cells in their immediate vicinity, but still remain alive, although they are usually not vigorous. Only the tips of the branches retain protoplasm, while the remainder of the hyphæ are completely vacuolated, with apparently no film of protoplasm next to the walls. The tips at this time usually are also vacuolated very distinctly and show very definite signs of approaching death. It seems clear, therefore, that whatever the intimate physiological relations between host and parasite, the death of the host cells is the direct result of the presence of the hyphæ, and that for some reason the hyphæ themselves succumb soon after.

The essential fact is that the fungus gains entrance in the same manner in susceptible and resistant forms, but acts differently thereafter. In susceptible forms it grows vigorously without seriously affecting the host cells for some time. In resistant forms, on the other hand, a very

rapid action results in the almost immediate death of the host cells. The degree of susceptibility is indicated to a certain extent by the rapidity of this action. The more resistant a form, the quicker are a few host cells in the immediate neighborhood of the invading hyphæ killed and the sooner does the fungus itself cease activity. The visual evidence is clear, but the exact interpretation of the results is more difficult. Marryat (5) considers that the hyphæ of *P. glumarum* in an uncongenial host starve on account of the death of the host cells. This may be the correct explanation, but there seems to be a very definite antagonism between the immune plant and the parasite, which may possibly require another explanation. The work of Ward (9–12) on *P. dispersa*, of Spinks (6) on *P. glumarum*, and of the writer (8) on *P. graminis* seems to indicate that immunity and resistance, especially when very marked, are quite independent of the nourishment of the plant, and although this does not necessarily establish the case, it would seem to point to a very fundamental antagonism. On the other hand, Comes (1) states that resistance in wheats is due to the acidity of cell sap. It seems clear, however, that plants nearly or quite immune to *P. graminis* exhibit the same phenomena in more extreme form as do partially or highly resistant forms.

SUMMARY

(1) When plants practically immune to *Puccinia graminis* are inoculated, the fungus gains entrance in a perfectly normal manner.

(2) After entrance the fungus rapidly kills a limited number of the plant cells.

(3) The fungus, after having killed the host cells in its immediate vicinity, seems unable to develop further.

(4) The relations between plant and parasite in partially resistant and almost totally immune plants are different in degree only.

(5) Hypersensitiveness of the host seems to be a common phenomenon not only among plants somewhat resistant to *P. graminis* but also among those almost totally immune to it.

LITERATURE CITED

(1) COMES, Orazio.
    1913. Della resistenza dei frumenti alle ruggini stato attuale della quistione e provvedimenti. *In* Atti R. Ist. Incoragg. Napoli, s. 6, v. 64, 1912, p. 421–441. Letteratura e note, p. 437–440.
(2) EVANS, I. B. Pole.
    1907. The cereal rusts. I. Development of their Uredo mycelia. *In* Ann. Bot., v. 21, no. 84, p. 441–466, pl. 40–43.
(3) FROMME, F. D.
    1914. Negative heliotropism of the urediniospore germ-tubes of Puccinia Rhamni. (Abstract.) *In* Phytopathology, v. 4, no. 6, p. 407–408.
(4) GIBSON, C. M.
    1904. Notes on infection experiments with various Uredineæ. *In* New Phytol., v. 3, no. 8, p. 184–191, pl. 5–6.

(5) MARRYAT, Dorothea C. E.

    1907. Notes on the infection and histology of two wheats immune to the attacks of Puccinia glumarum, yellow rust. *In* Jour. Agr. Sci., v. 2, pt. 1, p. 129–138, pl. 2.

(6) SPINKS, G. T.

    1913. Factors affecting susceptibility to disease in plants. *In* Jour. Agr. Sci., v. 5, pt. 3, p. 231–247, pl. 7.

(7) STAKMAN, E. C.

    1914. Relation between Puccinia graminis and host plants immune to its attack. (Abstract.) *In* Phytopathology, v. 4, no. 6, p. 400.

(8) ———

    1914. Study in cereal rusts: Physiological races. Minn. Agr. Exp. Sta. Bul. 138, 56 p., 9 pl.

(9) WARD, H. M.

    1888. Illustrations of the structure and life-history of Puccinia graminis, the fungus causing the "rust" of wheat. *In* Ann. Bot., v. 2, no. 6, p. 217–222, pl. 11–12 (col.).

(10) ———

    1901. The bromes and their rust-fungus (Puccinia dispersa). *In* Ann. Bot., v. 15, no. 59, p. 560–562.

(11) ———

    1902. Experiments on the effect of mineral starvation on the parasitism of the Uredine fungus, Puccinia dispersa, on species of Bromus. *In* Proc. Roy. Soc. London, v. 71, no. 469, p. 138–151, 4 fig., 4 tab.

(12) ———

    1902. On the relations between host and parasite in the bromes and their brown rust, Puccinia dispersa (Erikss.). *In* Ann. Bot., v. 16, no. 62, p. 233–315, 28 tab.

The outlines in this illustration were made on a level table by the aid of the camera lucida, using the Leitz achromatic objective No. 6 (4 mm.) and Huyghenian eyepiece Leitz No. IV (×10). The detail was studied under a magnification of 1,000.

Fig. 1.—Oats inoculated with *Puccinia graminis* from *Dactylis glomerata* after three generations on oats. Early infection stage, showing haustoria in the two epidermal cells to the right of the stoma and one in mesophyll cell. Host cells normal; infection normal and successful.

Fig. 2.—Same as figure 1, seven days after inoculation. Cell from edge of pustule area surrounded by hyphæ, but still normal.

Fig. 3.—Oats inoculated with *P. graminis hordei*, four days after inoculation. Piece of mycelium growing over cell; chloroplasts being destroyed; cell at right just being affected; haustorium in epidermal cell.

Fig. 4.—Same as figure 3. Infection thread growing over cell and destroying chloroplasts; normal cells on left.

Fig. 5.—Oats inoculated with *P. graminis tritici* four days after inoculation. Hypha growing over two cells, both of which have been killed; outlines of chloroplasts still showing faintly in second cell; cell to right of hypha becoming affected.

Fig. 6.—Oats inoculated with *P. graminis tritici*, 48 hours after inoculation. The cell on the left killed; outlines of chloroplasts still showing very faintly; cell on the right just becoming affected; tip of hypha dying.

PLATE XXVIII
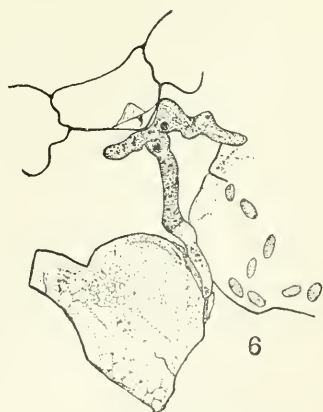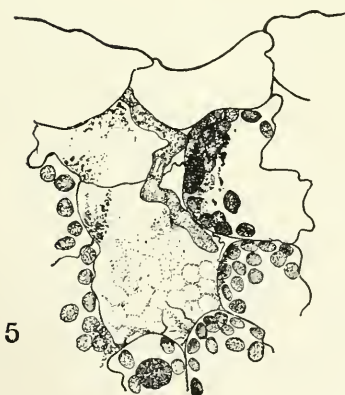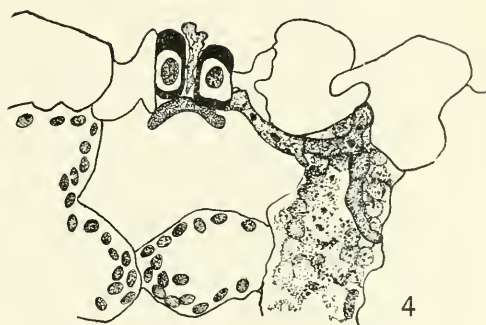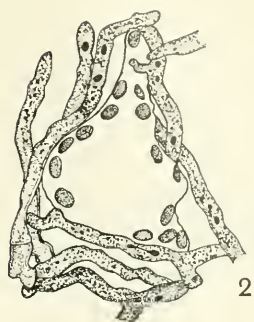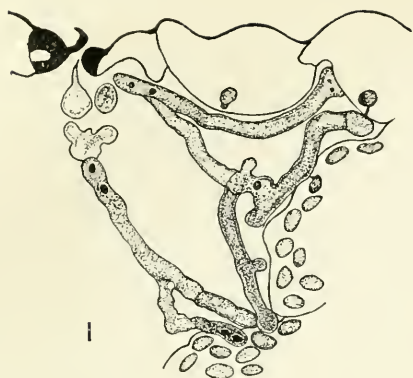
ANTAGONISM BETWEEN ANIONS AS AFFECTING BARLEY YIELDS ON A CLAY-ADOBE SOIL

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INTRODUCTION

In another publication (4)[1] the senior writer called attention to the results obtained on the antagonism between anions as affecting both the higher plants grown in soils and the bacterial flora in the soils. That brief statement constitutes, so far as we are aware, the first published observation on the existence of antagonism between anions in the case of plants grown in soils. Since the appearance in print of the statement just referred to, there has appeared the work of Miyake (10), which in reporting an elaborate series of experiments confirms the fact first enunciated by us as shown in the discussion below given. Our detailed results were withheld from publication pending such time as complete confirmation of them in repeated experiments in the same soil should make their appearance in print justifiable. These confirmatory data have now been completed, and we submit them below with such discussion as is deemed pertinent and necessary.

As may be inferred from the foregoing statements, there is no literature bearing directly on the subject, with the one exception of the paper noted. As is well known, however, a literature on the general principle of antagonism between ions is rapidly growing voluminous. To this the reader may gain access through Robertson's (14) excellent review of such investigations up to a recent date and through references given by the senior author in recent publications (3, 5) dealing with certain phases of the subject as applied to some of the soil bacteria. In a word, so much evidence has been adduced by various investigators in support of the existence of antagonism between ions for living organisms that we may now more properly speak of it as an established fact of great scientific interest and of practical import rather than as a theory, as heretofore.

In connection with the narrower subject of antagonism between anions, however, we reiterate that very little or nothing has been accomplished. One of the reasons for this is probably to be found in the repeated assertion of the ablest writers on the general subject in question to the effect that anions are of little, if any, importance in a consideration of antagonism between ions. Whatever be the cause, however, there are

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[1] Reference is made by number to "Literature cited," p. 217.

but three reported investigations in all branches of biology, so far as we are aware, which testify to the existence of antagonism between anions, as pointed out by the senior author in one of the papers above cited (3). Two of these were carried out with animals by Moore (11) and Neilson (12) and date back several years. The third is that by Miyake (10) above referred to, which appeared after nearly all of our work was completed and after the statement made by the senior author (4). Our experiments and results therefore constitute a pioneer effort in a virgin field. It is perhaps needless to add that the practical significance of these results when viewed from the standpoint of their bearing on the possible utility of alkali soils for crops is of great moment.

GENERAL DESCRIPTION OF THE EXPERIMENTS

The experiments were carried out in the greenhouse and the plants were grown in 8-inch paraffined pots. The soil employed was a clay-adobe type found on the campus of the University of California. The plant employed for the experiments was a selected strain of barley (*Hordeum* spp.). Quantities of soil equivalent to about 5 kg. on the basis of dry weight were mixed with the necessary salts, as indicated in the tables, and placed in the pots. The salts employed were the commonest of those of alkali lands—namely, sodium chlorid (NaCl), sodium sulphate (Na_2SO_4), and sodium carbonate (Na_2CO_3). For the purpose of strengthening the value of our results, two crops in succession were grown in the same pots, allowing the soil a rest period of about three months. Eight seeds were planted in each pot and the plants were later thinned to four to the pot. Water was supplied in quantities as nearly as possible approximating the amount necessary to maintain optimum moisture conditions. Duplicate pots were arranged to represent every one of the treatments. It may be frankly remarked here that results obtained in many of the duplicate pots were far from satisfying. We can not, however, see how these differences, which are brought about by inherent individual plant variations, and by slight differences in the physical condition of the soil in the different pots, could have been avoided. We do not consider our data, therefore, of absolute value and realize further that variations in the technique of our experiments might have yielded better results. Despite all that, however, mixtures of salts allowing the interaction of different anions have permitted, even under much higher osmotic pressures, so much better growth than in smaller concentrations of a single anion only that we feel fully justified in claiming our results to be proof of the undoubted existence of antagonism between anions. Indeed, that is the only claim put forward for our data, but as to its validity we can not see any objection. All other explanatory data are given in the following tables, which are discussed separately and more in detail.

TOXICITY OF THE SINGLE SALTS

It should be stated first in connection with the experiments here described that simultaneously with the series of pots containing salt mixtures of various kinds, in addition to the control plants of every series, several series of plants were also grown for the purpose of determining the degree of toxicity of every one of the salts employed. In these experiments the technique was exactly the same as that employed in the antagonism series. All explanatory data with respect to this added experiment which may be necessary to a comprehension of it are given in Table I, which is presented in full below, despite its very unsatisfactory nature as viewed from the standpoints above discussed.

TABLE I.—*Toxicity of sodium chlorid, sodium sulphate, and sodium carbonate for barley*

Experi-ment No.	Sodium chlorid.			Sodium sulphate.			Sodium carbonate.		
	Salt added.	Total produce.		Salt added.	Total produce.		Salt added.	Total produce.	
		First crop.	Second crop.		First crop.	Second crop.		First crop.	Second crop.
	Per cent.	*Gm.*	*Gm.*	*Per cent.*	*Gm.*	*Gm.*	*Per cent.*	*Gm.*	*Gm.*
I	0	4.30	12.70	0	4.30	12.70	0	4.30	12.70
I	0	3.60	12.85	0	3.60	12.85	0	3.60	12.85
2	0.10	6.70	5.60	0.10	8.40	9.30	0.05	7.00	15.95
2	.10	6.70	5.20	.10	7.20	8.75	.05	9.20	11.30
3	.15	4.30	5.90	.20	7.25	7.40	.10	8.00	9.40
3	.15	4.35	5.15	.20	6.55	9.25	.10	6.80	10.70
4	.20	3.50	5.35	.30	7.70	10.60	.15	8.20	7.80
4	.20	?6.40	6.50	.30	7.40	9.00	.15	8.40	9.75
5	.25	4.90	5.55	.40	4.65	7.30	.20	6.60	11.20
5	.25	3.25	5.55	.40	4.90	6.30	.20	9.40	9.35
6	.30	3.25	....	.50	5.50	5.98	.25	7.85	11.50
6	.30	3.30	....	.50	5.25	5.70	.25	9.10	10.32
7	.35	3.65	....	.60	2.08	6.30	.30	9.15	10.50
7	.35	3.30	....	.60	4.10	5.60	.20	9.50	14.90

Some very interesting points may be gleaned by even a cursory examination of Table I. These may be summarized as follows:

(1) Very little, if any, toxicity is manifested by any of the salts in the first crop.

(2) Decided stimulation is evident in the lowest concentrations employed of every one of the salts in the first crop.

(3) No concentration of sodium carbonate employed gave anything but strong stimulation in the first crop.

(4) Stimulation was almost at a standstill in the sodium-sulphate series at and above the 0.4 per cent concentration in the first crop.

(5) Stimulation was at a standstill in the sodium-chlorid series at and above the 0.15 per cent concentration in the first crop.

(6) The total yields in the second crop throughout are much larger than in the first crop, indicating almost certainly an improvement in the soil and climate during the growth of the first crop and the next period between the two crops.

(7) The toxicity of sodium chlorid and sodium sulphate is plainly discernible in the second crop, even at the lowest concentrations employed. Note the difference between this statement and statement 1.

(8) In the case of sodium carbonate it seems probable that a slight toxicity exists also, even at the lowest concentration. Such toxicity is not nearly so marked, however, as in the cases of the two other salts.

(9) It is remarkable to note the high yields obtained in the sodium-carbonate series of the second crop, as well as the uniformly poor agreement between duplicate pots in the series.

(10) In general, it is a striking fact not easily accounted for that once toxicity does manifest itself it does not seem to become notably more acute as the quantity of salt present increases.

The behavior of the cultures at the lowest concentrations in the first crop is probably to be attributed to an improvement in the physical, chemical, and biological condition of the heavy clay-adobe soil through the salt applications. At the same time the control soils had improved during the growth of the first crop and especially during the period intervening between the two crops much more markedly than the soils treated with the smallest quantities of salts. This improvement was doubtless wrought by good crumb formation in the soil through alternate wetting and drying at first and later by thorough drying during the period of rest. After such improvement, therefore, the control pots showed marked superiority over the pots nearest them in the series which during the same period had changed but slightly, except in the case of the sodium-carbonate series. While, therefore, the yields of the control pots had trebled in the second crop, they remained practically the same at the lowest sodium-chlorid and sodium-sulphate concentrations. It remains true, however, that generally the yields of the second crop were superior to those of the first, a fact to be attributed, in addition to the above-mentioned causes, to the seasonal and climatic differences obtaining between the periods of growth of the two crops.

ANTAGONISM BETWEEN SODIUM CHLORID AND SODIUM SULPHATE

Table II gives the results obtained in two successive crops in the series of experiments on antagonism between sodium chlorid and sodium sulphate. The toxic quantity of sodium chlorid used throughout was 0.2 per cent, and sodium sulphate was added in varying quantities up to 0.5 per cent.

TABLE II.—*Results of experiments on antagonism between sodium chlorid and sodium sulphate*

Experiment No.	Sodium chlorid.	Sodium sulphate.	First crop.				Second crop.			
			Tops.	Roots.	Total.	Total average.	Tops.	Roots.	Total.	Total average.
	Per cent.	Per cent.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
1	0	0	3.00	0.60	3.60	} 4.10	10.20	2.50	12.70	} 12.77
1	0	0	3.50	1.10	4.60		10.50	2.35	12.85	
2	0.20	0	4.00	.65	4.65	} 5.32	7.20	.60	7.80	} 7.25
2	.20	0	5.40	.60	6.00		5.90	.80	6.70	
3	.20	0.10	4.10	.15	4.25	} 3.77	8.30	1.00	9.30	} 9.45
3	.20	.10	3.10	.20	3.30		8.50	1.10	9.60	
4	.20	.15	4.30	.45	4.75	} 4.72	7.80	.95	8.75	} 9.37
4	.20	.15	4.50	.20	4.70		9.00	1.00	10.00	
5	.20	.20	4.80	.35	5.15	} 5.27	10.00	1.30	11.30	} 10.52
5	.20	.20	5.20	.20	5.40		9.00	.75	9.75	
6	.20	.25	2.70	.25	2.95	} 2.55	9.40	1.00	10.40	} 9.60
6	.20	.25	2.00	.15	2.15		7.80	1.00	8.80	
7	.20	.30	2.80	.20	3.00	} 2.32	9.00	1.23	10.23	} 10.06
7	.20	.30	1.60	.05	1.65		8.30	1.60	9.90	
8	.20	.35	1.70	.15	1.85	} 1.80	11.20	.95	12.15	} 11.77
8	.20	.35	1.60	.15	1.75		10.30	1.10	11.40	
9	.20	.40	2.80	.20	3.00	} 2.27	8.30	.68	8.98	} 9.68
9	.20	.40	1.40	.15	1.55		9.40	.98	10.38	
10	.20	.45	2.00	.30	2.30	} 2.57	7.00	.60	7.60	} 8.66
10	.20	.45	2.60	.25	2.85		8.80	.92	9.72	
11	.20	.50	1.60	.10	1.70	} 1.70	7.30	.84	8.14	} 9.17
11	.20	.50	....	....	....		9.20	1.00	10.20	

It is plain that in the first crop there can have been only the slightest antagonism, if any. It is true, however, that the medium of growth does not seem to have become seriously impaired through the addition of sodium sulphate to the constant quantity of sodium chlorid up to and including 0.2 per cent of sodium sulphate. After that, a very definite depression in growth and yield is noticeable as more sodium sulphate is added, indicating increased toxicity at combinations of 0.2 per cent of sodium chlorid and 0.25 per cent of sodium sulphate, and above.

Quite different conditions confront us in those parts of Table II devoted to the results of the second crop. We note here, as in the foregoing toxicity series (Table I), not only a marked depression in yield in experiment 2 resulting from the presence of 0.2 per cent of sodium chlorid but also a marked improvement in the yield when sodium sulphate is added to the common salt. Thus, while we obtain an average yield of 7.25 gm. of dry matter (tops and roots) when 0.2 per cent of sodium chlorid is present as against 12.77 gm. in the control pots, the yield is increased to 9.45 gm. when 0.1 per cent of sodium sulphate is present with 0.2 per cent of sodium chlorid, and is still further augmented to 10.52 gm. by the presence of 0.2 per cent of sodium sulphate with sodium chlorid. To be sure, as was remarked above, much irregularity exists

among the figures given; nevertheless, it can not be denied that through-
out the series all the salt mixtures yield far better results than the 0.2
per cent of sodium chlorid alone. Indeed, only in one salt mixture (one
of the pots of experiment 10) does the yield fall as low as in the
duplicate pots of experiment 2. In one case, No. 8, the total yield
of dry matter, while not equal to that of the controls, is very near the
latter, despite the fact that the soil contains 0.55 per cent of total salts.
Yet, in the case of No. 2, with considerably less than half the amount of
salt present, the yield is depressed approximately 40 per cent below that
of the control pots. In the face of such data, erratic as they seem in
some respects, there can be no denial of the existence of antagonism
between anions. This record is the first, except the preliminary note (4)
referred to above, establishing the existence of antagonism between
anions for the higher plants *when the latter are grown in normal soils.*

ANTAGONISM BETWEEN SODIUM CHLORID AND SODIUM CARBONATE

In this series there was again employed the constant toxic quantity of
0.2 per cent of sodium chlorid throughout. The varying antagonistic
agent of the last series, however (sodium sulphate), was here supplanted
by sodium carbonate. Other explanatory data are recorded in Table III.

TABLE III.—*Results of experiments on antagonism between sodium chlorid and sodium carbonate*

Experiment No.	Sodium chlorid.	Sodium carbonate.	First crop.				Second crop.			
			Tops.	Roots.	Total.	Total average.	Tops.	Roots.	Total.	Total average.
	Per cent.	Per cent.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
1	0	0	3.00	0.60	3.60	} 4.10	10.20	2.50	12.70	} 12.77
1	0	0	3.50	1.10	4.60		10.50	2.35	12.85	
2	0.20	0	4.00	.65	4.65	} 5.32	7.20	.60	7.80	} 7.25
2	.20	0	5.40	.60	6.00		5.90	.80	6.70	
3	.20	0.05	4.80	.40	5.20	} 5.90	7.80	.73	8.53	} 8.19
3	.20	.05	6.20	.40	6.60		7.30	.55	7.85	
4	.20	.10	4.80	.20	5.00	} 5.17	11.40	.80	12.20	} 11.25
4	.20	.10	5.20	.15	5.35		9.20	1.10	10.30	
5	.20	.15	....	....	....	} 2.55	12.00	.30	12.30	} 12.35
5	.20	.15	2.50	.05	2.55		11.50	.90	12.40	
6	.20	.20	2.80	.05	2.85	} 3.25	13.50	.65	14.15	} 12.93
6	.20	.20	3.60	.05	3.65		10.20	1.50	11.70	
7	.20	.25	4.80	.20	5.00	} 3.87	9.00	.35	9.35	} 10.23
7	.20	.25	2.70	.05	2.75		10.20	.90	11.10	
8	.20	.30	(a)	(a)	(a)	} (a)	7.70	.50	8.20	} 10.50
8	.20	.30	(a)	(a)	(a)		12.30	.50	12.80	

a No growth.

Studying the data of Table III, evidence of only slight antagonism in
the first crop is again seen. It should be noted, however, that, slight as
it is, it is much more definite than in the case of the foregoing series.

On the other hand, the increased toxicity which follows the points of antagonism is much more sharply marked in the series immediately under discussion, and only 0.15 per cent of sodium carbonate need be added to 0.2 per cent of sodium chlorid to depress the yield to the point to which it takes an addition of 0.25 per cent of sodium sulphate to 0.2 per cent of sodium chlorid to depress it.

In the case of the second crop, data of very similar nature to those of the foregoing series are noted. The addition of even small quantities (0.05 per cent) of sodium carbonate to 0.2 per cent of sodium chlorid is instrumental in bringing about noticeably better growth, whereas markedly higher results are obtained when amounts of sodium carbonate equivalent to 0.1 per cent of the dry weight of the soil are added to 0.2 per cent of sodium chlorid. The maximum yield is obtained in No. 6, in which the total dry matter produced is even greater than that of the control pots. Even the addition of 0.3 per cent of sodium carbonate produces marked antagonism to the sodium chlorid and allows a good yield. The data in this series are therefore even more emphatically in support of the existence of antagonism between anions than those of the foregoing series involving the interaction of sodium chlorid and sodium sulphate. Thus, again, an increase in the total alkali content of the soils from 0.2 to 0.5 per cent by the addition of 0.3 per cent of sodium carbonate to 0.2 per cent of sodium chlorid, so far from rendering the soil a much poorer medium for plant growth, has made it even better than the control soils containing no salt, and nearly twice as good a producer as the same soil containing the same quantity of sodium chlorid but no sodium carbonate.

ANTAGONISM BETWEEN SODIUM CARBONATE AND SODIUM SULPHATE

The arrangement of this series was similar to that of the preceding series, sodium carbonate, however, being used as the constant toxic salt and sodium sulphate being used in varying quantities for purposes of antagonism. The results follow in Table IV.

This is the only series of those submitted in this paper which gives no proof of antagonism between anions when two salts are mixed. Apparently there seems to be antagonism in this series not only in the case of the second crop, as in the foregoing series, but also in the case of the first crop. In reality, however, this is not so, as can be seen by an examination of the data submitted in Table I. From the latter we see that the assumed toxicity of culture medium 2 of Table IV is greater than that of any other of the toxicity series for sodium carbonate, even where twice as much sodium carbonate is employed. While, therefore, there may be an actual antagonism between sodium carbonate and sodium sulphate, the evidence of it in Table IV is absolutely untrustworthy, and largely for the reason that no quantity of sodium carbonate employed has actually been shown to be definitely toxic to barley in the clay-adobe

soil. This is in striking contrast to the results given in Tables II and III, which are based on toxic properties of given quantities of sodium chlorid that are shown to be definite and constant in Table I.

TABLE IV.—*Results of experiments on antagonism between sodium carbonate and sodium sulphate*

Experiment No.	Sodium carbonate.	Sodium sulphate.	First crop.				Second crop.			
			Tops.	Roots.	Total.	Total average.	Tops.	Roots.	Total.	Total average.
	Per cent.	Per cent.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
1	0	0	3.00	0.60	3.60	4.10	10.20	2.50	12.70	12.77
1	0	0	3.50	1.10	4.60		10.50	2.35	12.85	
2	0.15	0	5.40	1.20	6.60	6.30	7.70	1.05	8.75	8.27
2	.15	0	5.00	1.00	6.00		6.90	.90	7.80	
3	.15	0.10	4.00	1.20	5.20	6.20	6.50	.95	7.45	8.66
3	.15	.10	6.60	.60	7.20		9.00	.87	9.87	
4	.15	.15	7.20	.45	7.65	7.65	....	....	....	....
4	.15	.15	....	....	....					
5	.15	.20	5.50	1.20	6.70	7.30	9.30	1.20	10.50	9.85
5	.15	.20	7.00	.90	7.90		8.00	1.20	9.20	
6	.15	.25	4.50	.65	5.15	4.93	9.00	1.30	10.30	9.60
6	.15	.25	4.70	....	4.70		8.00	.90	8.90	
7	.15	.30	4.50	.50	5.00	5.45	9.50	.80	10.30	9.97
7	.15	.30	5.50	.40	5.90		8.50	1.15	9.65	
8	.15	.35	6.30	.50	6.80	6.48	9.00	1.45	10.45	10.63
8	.15	.35	5.50	.65	6.15		10.20	.70	10.90	
9	.15	.40	4.00	.20	4.20	4.95	9.00	.95	9.95	11.07
9	.15	.40	5.50	.20	5.70		11.00	1.20	12.20	
10	.15	.50	6.80	.10	6.90	5.55	....	....	....	....
10	.15	.50	4.00	.30	4.30					

ANTAGONISM BETWEEN CALCIUM SULPHATE AND SODIUM SULPHATE

While this paper was intended primarily to deal with results obtained with the interaction of anions of the common alkali salts, the antagonism between which has been above established, other interesting factors relating thereto deserve brief consideration here. Questions naturally arise in connection with such work involving the relative efficiency of different salts in counteracting a given toxic salt. Is it, for example, reasonable, on the basis of Loeb's experiments (7, 8), to suppose that bivalent ions like those of calcium would be more efficacious in the antagonism of salts with a monovalent cation than another salt with a monovalent cation? This is a practical question of great importance, so far as the subject under discussion here is concerned. For example, it is of importance for us to know whether sodium sulphate, which occurs in such large quantities in our alkali soils, at times singly and at times together with other salts, can be prevented from reacting deleteriously to plant growth in those soils by the application of gypsum. The latter salt of calcium is now much used in practice for purposes of counteracting the

bad effects of sodium carbonate ("black" alkali) and also as a stimulant to certain legumes which are grown for forage. As was demonstrated by Hilgard (1, p. 457–458) in proposing the use of gypsum for the last-named purpose, the following reversible reaction takes place, which accounts for the beneficial effect of the gypsum for reasons too well known to need repetition here.

$$Na_2CO_3 + CaSO_4 = Na_2SO_4 + CaCO_3$$

However, Hilgard also observes (1, p. 458), "of course, gypsum is of no benefit whatever on soils containing no 'black' alkali, but only ('white') Glauber's and common salt." The finality of this expression only emphasizes again what has been noted so often before—namely, the danger that lurks in positive statements, at least in the inexact sciences, no matter how certain their correctness may appear at the time. In the light of the more recent information on antagonism between ions, one would not subscribe to the statement just quoted. It was, indeed, because of the rapidly accumulating data on antagonism between salts that we were led to doubt the finality of Hilgard's statement and to learn by direct experiment the facts in the case.

Accordingly an experiment similar to those above described was arranged, except that sodium sulphate was used in constant toxic quantity of 0.4 per cent and calcium sulphate ($CaSO_4$) in varying quantities to determine whether any interaction occurs between these salts which proves of value to plant growth in such soils as those here described. It will be remembered that we are dealing here with the same anion but with different cations, one of the latter having a higher valence than the others. The evidence of antagonism given above was obtained with the same cation but with different anions. Other information respecting the mode of arrangement of the experiment, as well as the results thereof, is given in Table V.

Table V not only shows the incorrectness of Hilgard's view but evidences most emphatically that calcium sulphate is a very efficient substance for the purpose of preventing the toxicity of sodium sulphate. In this series we have antagonism in the first crop as well as in the second, a phenomenon only very dubiously noted in the foregoing antagonism series. That the relatively large additions of calcium sulphate should continue, like some of the smaller additions, to show an effect antagonistic to sodium sulphate is not surprising, inasmuch as gypsum is a relatively insoluble salt and would therefore not be expected to cause an increased toxicity when added to another salt, as would be the case with the more soluble salts above studied. Two facts are shown in Table V which are very difficult to explain. One is the different point as regards the concentration of salts at which the most marked antagonism occurs in the two crops, and the other is the behavior of small amounts of gypsum

as compared with the larger amounts in the first crop. We are unable to explain satisfactorily why 0.1 per cent of calcium sulphate should in the first crop render 0.4 per cent of sodium sulphate much more toxic than it is alone, and in the second crop virtually inhibit its toxicity. This can not be accidental, since the same result is obtained in another set of duplicate pots differing from those just described only in containing 0.15 per cent of calcium sulphate instead of 0.1 per cent. Similar results have been obtained by the senior writer and Mr. P. S. Burgess in other investigations (6), but they remain as difficult as ever to explain. This case is particularly troublesome, since the same concentration of salts in the same pot gives practically no crop the first season and a very good crop the second season. In the first case the salts show increased toxicity when calcium sulphate is added to sodium sulphate, whereas a few months later the maximum of antagonism is noted with the same salt mixture in the same soil and pot.

TABLE V.—*Results of experiments on antagonism between calcium sulphate and sodium sulphate*

Experiment No.	Sodium sulphate.	Calcium sulphate.	First crop.				Second crop.			
			Tops.	Roots.	Total.	Total average.	Tops.	Roots.	Total.	Total average.
	Per cent.	*Per cent.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	0	0	3.00	0.60	3.60	} 4.10	10.20	2.50	12.70	} 12.77
1	0	0	3.50	1.10	4.60		10.50	2.35	12.85	
2	0.40	0	4.30	.35	4.65	} 4.77	6.70	.60	7.30	} 6.80
2	.40	0	4.50	.40	4.90		5.80	.50	6.30	
3	.40	0.10	1.80	.40	2.20	} 2.30	9.20	1.90	11.10	} 11.00
3	.40	.10	2.10	.30	2.40		9.00	1.90	10.90	
4	.40	.15	1.30	.30	1.60	} 2.23	9.60	2.45	12.05	} 10.88
4	.40	.15	2.30	.55	2.85		7.70	2.00	9.70	
5	.40	.20	6.20	.70	6.90	} 6.40	......	......	......	} ....
5	.40	.20	5.60	.30	5.90		......	......	......	
6	.40	.25	5.00	.70	5.70	} 6.10	7.00	1.30	8.30	} 8.70
6	.40	.25	5.80	.70	6.50		8.00	1.10	9.10	
7	.40	.30	6.90	.70	7.60	} 7.30	9.00	2.35	11.35	} 10.73
7	.40	.30	6.00	1.00	7.00		8.40	1.70	10.10	
8	.40	.35	5.40	1.40	6.80	} 6.85	7.20	1.55	8.75	} 10.45
8	.40	.35	6.00	.90	6.90		11.40	.75	12.15	
9	.40	.40	5.50	.60	6.10	} 5.45	6.20	1.45	7.65	} 8.55
9	.40	.40	4.00	.80	4.80		8.00	....	9.45	
10	.40	.45	7.60	1.00	8.60	} 7.50	8.60	2.25	10.85	} 10.85
10	.40	.45	5.00	1.40	6.40		......	......	......	
11	.40	.50	4.70	.80	5.50	} 5.75	8.60	2.25	10.85	} 11.40
11	.40	.50	5.30	.70	6.00		9.70	....	11.95	

Whatever the cause of this puzzling fact may be, the results given in Table V leave no room for doubt as to the power of gypsum to antagonize the toxic effects of Glauber salt (sodium sulphate) in a clay-adobe soil, barley being the plant grown. It must also be noted in this connection

that, at least so far as some seasons are concerned, small quantities of gypsum are as efficient for the purpose as larger quantities, if not more so. Indeed, this would seem to reply to one of the questions above raised as to the rôle of valence of ions in antagonism. It appears that small quantities of calcium are as efficacious in antagonism to other ions as large quantities of sodium or other univalent ions—or even more so.

GENERAL DISCUSSION OF THE EXPERIMENTS

Several questions arise in connection with the discussion of the foregoing experiments which deserve brief attention here.

GRAIN YIELDS AND ROOT PRODUCTION AS RELATED TO ANTAGONISM

Thinking that it might be possible to correlate grain or straw yields with antagonism between ions or with the lack thereof, we proceeded to determine separately from the total weight of the tops of the barley plants the weight of the grain produced. No regularity in the production of grain with respect to soil treatment was found. At times the treatment which yielded the largest amount of dry matter would also be found to give the highest grain yields, but very frequently no such relation could be established. If anything general could be stated with reference to the grain yields of the barley plants, it would be that grain production was nearly uniform throughout the series in any given season. No significance can be found, therefore, to judge from our figures, in the grain yields as criteria of absence or presence of antagonism.

The case is not similar with respect to root production. No matter how marked antagonism may be, as judged by the production of total dry matter, root development from the absolute standpoint is always markedly depressed in the presence of salts in the soil. To be sure, the root production is often improved when total yields are increased, but never in the same proportion, and it will be noted throughout in the tables that the root production is always largest in the control pots. Moreover, in the case of the root growth as in that of grain yield, we find great irregularity, for much of which we are unable to account.

COMPARISON OF OUR RESULTS WITH THOSE OF OTHER INVESTIGATORS

As stated above, the only other results, so far as we are aware, which have been obtained in antagonism work with anions are those of Miyake (10), which were published after our preliminary statement had appeared. Even Miyake's work, however, gives no results of the antagonism between anions as noted in soil cultures, for all his experiments of this kind were carried out in solutions. Nevertheless, the general nature of the work of the Japanese investigator may here be mentioned for comparison with ours and in confirmation of the latter. Miyake found that for the rice plant (*Oryza sativa*) grown in culture solutions antagonism is apparent

between the $-Cl$, $-SO_4$, and $-NO_3$ ions; that such antagonism, however, is not as marked as that between cations; and that the antagonism of one ion to another—for example, $-SO_4$ to $-Cl$—may be greater than under opposite conditions—e. g., $-Cl$ to $-SO_4$. Likewise, as between $-NO_3$ and $-SO_4$, the former ion has the superior power to neutralize the toxic effects of the other. In so far as antagonism between the anions is observed by Miyake, he confirms by less striking examples in culture solutions what we have shown takes place in soil cultures. We can not agree, however, on the basis of our results that antagonism between anions is more feeble than that between cations. The differences found by Miyake in the power of two anions to counteract mutually each other's toxicity has been pointed out in relation to the nitrifying bacteria by the senior writer and Burgess in another place (5).

Our great caution in pointing out differences between soil and solution cultures is explained in several different publications, some of which are cited in this paper. It must be remembered, moreover, that on the basis of direct comparison of the soil and solution cultures Kearney and Cameron (2) pointed out several years ago the very material differences obtaining between all phases of salt effects in solutions and in field experiments. The importance of this point in investigations of salt effects on living organisms which are intended ultimately for practical application can not be overemphasized.

MAINTENANCE OF THE ALKALI CONTENT IN THE EXPERIMENTAL SOIL

In anticipation of queries with reference to the maintenance of the original "alkali" concentration in the soils described throughout the experiment the following statement is made. The irrigation was so carried out that drainage from the soils was never noted. In other words, enough water was supplied to provide the plants with all the moisture necessary, but no excess was employed. By keeping glazed plates beneath the pots it was possible to note constantly the lack of percolation from the pots. Moreover, samples of soil were removed from the pots at the end of the second season of growth and analyzed for "alkali." It was always possible to recover all or very nearly all the sodium chlorid and sodium sulphate that had been originally added. Sometimes the quantity recovered showed slightly less and at other times slightly more than was added. These irregularities are doubtless due to the slightly imperfect mixing of the salts with the soil or are errors inherent in the method of determination employed.

Quite the contrary was true, however, of the pots receiving sodium carbonate. Not only was it impossible to recover all the sodium carbonate that had been added at the commencement of the experiment, but it was actually possible to recover very little of that salt, the highest percentage recovered being about 25 per cent of the amount first added. This would

perhaps in part explain the peculiar behavior of this salt in the toxicity series which we have discussed, as well as its behavior in the antagonism series; for small quantities of sodium carbonate, which evidently are all that can be counted on to remain in the soil any length of time, might well act as stimulants rather than as toxic agents. This view is to be considered in conjunction with those above discussed on the behavior of sodium carbonate. However that may be, the slight recovery of this salt effected by us from the treated pots would seem to support even more strongly the view expressed above, in which an analogy is drawn between the behavior of sodium carbonate and that of magnesium carbonate ($MgCO_3$) as first explained by MacIntire (9).

PRACTICAL CONSIDERATION OF THE EXPERIMENTS

It appears plain, in view of the results of Miyake (10) and ourselves (3–6), that the establishment of the existence of antagonism between anions is invested with at least a certain measure of practical importance. In the State of California, as well as in several other of our Western States, very large acreages of land are to be found in which the predominance of one salt, frequently Glauber salt or common salt, makes impossible successful cropping. It would appear from the above results that it would not be a difficult matter to establish a mode of treatment which would involve the neutralization of the toxic effects of any one or even two of the alkali salts by another alkali salt. Thus, we frequently find soils containing, besides small quantities of other salts, about 0.5 per cent of sodium sulphate. It is clear that in a heavy soil, at least by additions of gypsum at the rate of about 2 tons to the acre or common salt in smaller quantity, we could change the soil from a very poor into a normally producing one, despite the fact that we have very considerably increased the total salt content thereof.

THEORETICAL CONSIDERATIONS OF THE EXPERIMENTS

Several questions of interest, at present merely in their theoretical aspects, arise from the foregoing discussion and the results which form the basis thereof.

The differences in yields of two successive seasons in the same soils and pots are probably to be largely, though not entirely, attributed to temperature and atmospheric variations. To judge from the data submitted in the tables, the change in the soil's condition from one season to another has operated only in a minor way toward crop improvement. On the other hand, it is not impossible to regard the results as indicative of the opposite condition if particularly great stress is laid on the yields of the control pots during the second season.

The causes of antagonistic action still remain the topics of investigation most difficult of solution. Our results can only point indirectly to possible solutions of this important question. It is, however, interesting to

note that the nitrifying powers of soils were always found to be far superior in those containing mixtures of salts favorable to barley growth. There appears to be a direct relation, therefore, between the nitrate-nitrogen supply and barley growth, as pointed out by the senior writer elsewhere (4); and, further, in view of our specific tests in connection with the experiments under discussion and others, there seems to be a direct relationship between the qualitative and quantitative salt relationships in a soil and its nitrifying power. Is it not just possible, therefore, that in one important respect at least antagonism between ions in soils is attributable to the improved conditions brought about in the nitrate supply? One important question, however, would still remain: Why does a certain salt combination improve the nitrifying power of a soil? This question may perhaps be solved by methods now being employed by Loeb (7, 8) and Osterhout (13), but the answer thereto still appears to be very remote.

The puzzling fact, which has been referred to above, of the difference in effect of a single salt on barley in the same soil in two successive crops permits some interesting theoretical considerations. It appears possible that the stimulating effect noted in the first crop as proceeding from the addition of the lowest quantity of every one of the salts is to be attributed indirectly to a physical improvement in the heavy clay-adobe soil for reasons too well known to soil scientists to need discussion here; in other words, the yield of dry matter obtained with additions of 0.1 per cent of sodium chlorid and 0.1 per cent of sodium sulphate is to be regarded as representing the algebraic sum of the improvement in the soil's physical condition through the action of the salt and the depression in growth through direct influences on the barley plant and indirectly on the soil bacteria. Assuming, however, that the improvement wrought in the soil's physical condition is a greater factor for crop improvement in this case than the last-named effects are for the depression of plant growth, one would naturally expect that the results of the interaction of the two phenomena must be to produce a larger crop in salt-treated soil than is produced in the untreated control soil. The next question will be, therefore, How can one account for the remarkable improvement in the yield of the control soil in the second crop? This, it appears to us, is explicable on the basis of a gradual improvement in the control soil during the growth of the first crop through root action and appreciable changes in contraction and expansion, resulting in better crumb structure; but more completely through a physical improvement of the thoroughly mixed, dry control soil, which is allowed to bake in the loose condition for three months or more between the two crops. The crop produced in the control pots during the second season therefore has all the advantages of physical soil improvement, or many of them, possessed by the salt-treated soil during the first season, and in addition is free from disadvantages introduced by the salt, as explained above. On the basis of

such conceptions, which we offer as a tentative explanation, it seems easy to see why improvement is at first wrought in the clay-adobe soil by the sodium-chlorid and sodium-sulphate treatment and later why a depression is produced by the same salt treatment in the second crop. Actually the toxic effect appears to be there from the beginning but is obliterated by the good effects on the physical condition of the soil wrought by the salt. Given a good physical condition in the soil, however, the toxic effect of the salt becomes easily manifest.

Another matter of interest arises in connection with the behavior of sodium carbonate. It will be noted in the discussion of the last paragraph that the effects of sodium chlorid and sodium sulphate only are considered and not sodium carbonate. This is done advisedly, since an examination of Table I will show that sodium carbonate acts in a different way from the other salts, especially in the second crop. This, it would appear to us, is to be explained on the basis of the distinctive effects of that salt in a chemical and physiological way. As explained by the senior writer in other publications sodium carbonate is a stimulant to ammonification and a deterrent to nitrification. It is possible, therefore, and this is offered merely as a speculation, that stimulated ammonification may result in the direct absorption by the barley of ammonia instead of nitrate; and, if ammonia can be readily assimilated by the barley plant, the large amount of ammonia produced by the soils treated with sodium carbonate should cause marked vegetative development owing to better nitrogen feeding; hence, more dry matter. Moreover, other important considerations enter into this problem. Sodium carbonate is readily transformed into other forms when it is mixed with the soil and carbon dioxid (CO_2) is given off in accordance with the same principle which MacIntire et al. (9) have shown to apply to magnesium carbonate when it is mixed with the soil. We should thus obtain other compounds of sodium, probably silicates of that element, which would react differently from sodium carbonate. The marked solvent effect on soil minerals, moreover, which is possessed by this salt would seem to indicate a larger supply of available plant food in the soils treated with this salt and, hence, better plant growth. All of these beneficial effects of sodium carbonate could far outweigh its detrimental effects on the physical condition of the soil and yield the results noted. Indeed, in our more recent work we have obtained results that render questionable the great powers attributed to this salt in destroying the physical condition of all soils or of affecting plants deleteriously.

Pursuant to the last-mentioned idea, it is not out of place here to state in general that the direct toxic effect on plants of the "alkali" salts under consideration has been much exaggerated. The question of alkali tolerance by plants would appear in the light of our recent experiments to resolve itself really into one of alkali tolerance by soils. It is the effect of salts on the latter that is more likely to result seriously for plants than the

interference of salts directly with the normal functioning of plants. It may further be added that our experiments convince us also that even the effects of salts on soils are of an indirect nature, and, with the exception of cases of soils containing 0.75 per cent of total salts or more, they do not offer very serious practical problems in reclamation. This last remark is offered tentatively only as a hope for the practice of alkali-land reclamation, but at present seems well supported in fact.

The curious behavior of gypsum in the first crop, Table V, may be explained from the theoretical standpoint as a result of the fixation of bases, which in turn would change the nature of the soil solution. For example, a relatively small quantity of gypsum, which relatively is a slightly soluble salt, would set free by exchange of bases a certain amount of magnesium in the soil solution. Magnesium, as has been shown by several investigators, is detrimental in some concentrations to plant growth. This might therefore point to a direct toxic effect of magnesium resulting from an application of calcium sulphate. When, however, much more of the latter salt is added to the soil, an excess of calcium is introduced which neutralizes the toxic effects of the magnesium as well as of the sodium sulphate present, and the growth of barley is very much improved. This is offered merely as a speculation of interest and perhaps of significance in connection with the phenomenon noted in the series given in Table V. We recognize in some ways the inadequacy of the foregoing explanation and are not unaware of the flaws in the theory, but we feel that it may lead finally to an explanation of the facts noted.

In concluding the discussion, we desire to state that many other considerations of a theoretical nature enter into the subject of antagonism between anions in soils. The latter are such complicated media, however, and involve so many changes of an intensely complicated nature, that it would be impossible to discuss all these questions here.

SUMMARY

Results are given above which establish for the first time, so far as we are aware, the existence of antagonism between anions in a clay-adobe soil for barley as follows:

(1) Antagonism is shown between sodium chlorid and sodium sulphate and between sodium chlorid and sodium carbonate in the second crop. None is shown in the first crop.

(2) Slight antagonism is shown between sodium carbonate and sodium sulphate in the first crop. It is questionable whether any exists at all in the second crop.

In subsidiary experiments the following points are established in addition to those named above.

(1) Marked antagonism exists in both the first and second crop between sodium sulphate and calcium sulphate in soil cultures. This has not been considered possible hitherto by Hilgard.

(2) In testing the toxicity of single alkali salts it is found that 0.1 per cent each of sodium chlorid and sodium sulphate stimulates barley in the first crop and reacts poisonously to it in the second crop.

(3) Sodium carbonate does not manifest toxicity, but, on the contrary, shows stimulation even up to concentrations equal to 0.3 per cent of the dry weight of the soil.

LITERATURE CITED

(1) HILGARD, E. W.
    1910. Soils ... 593 p., illus. New York, London.
(2) KEARNEY, T. H., and CAMERON, F. K.
    1902. Some mutual relations between alkali soils and vegetation. U. S. Dept. Agr. Rpt. 71, 78 p.
(3) LIPMAN, C. B.
    1913. Antagonism between anions as affecting ammonification in soils. *In* Cent. Bakt. [etc.], Abt. 2, Bd. 36, No. 15/18, p. 382–394, 3 fig.
(4) ———
    1914. The theory of antagonism of salts and its significance in soil studies. *In* Soc. Prom. Agr. Sci. 34th Ann. Meeting, 1913, p. 33–40.
(5) ——— and BURGESS, P. S.
    1914. Antagonism between anions as affecting soil bacteria. *In* Cent. Bakt. [etc.], Abt. 2, Bd. 41, No. 11/17, p. 430–444, 6 fig.
(6) ——— ———
    1914. The effect of copper, zinc, iron lead salts on ammonification and nitrification in soils. *In* Univ. Cal. Pub. Agr. Sci., v. 1, no. 6, p. 127–139.
(7) LOEB, Jacques.
    1912. Antagonistic action of electrolytes and permeability of the cell membrane. *In* Science, n. s., v. 36, no. 932, p. 637–639.
(8) ———
    1914. Is the antagonistic action of salts due to oppositely charged ions? *In* Jour. Biol. Chem., v. 19, no. 3, p. 431–443.
(9) MacINTIRE, W. H., WILLIS, L. G., and HARDY, J. I.
    1914. The nonexistence of magnesium carbonate in humid soils. Tenn. Agr. Exp. Sta. Bul. 107 (Tech. Ser. 3), p. 151–202, 4 fig.
(10) MIYAKE, Koji.
    1914. Influence of the salts common in alkali soils upon the growth of rice plants. *In* Jour. Col. Agr. Tohoku Imp. Univ. Sapporo, Japan, v. 5, pt. 8, p. 241–319.
(11) MOORE, Anne.
    1902. On the power of Na_2SO_4 to neutralize the ill effects of NaCl. *In* Amer. Jour. Physiol., v. 7, no. 3, p. 315–319.
(12) NEILSON, Hugh.
    1902. Further experiments on the antitoxic effect of ions. *In* Amer. Jour. Physiol., v. 7, no. 5, p. 405–408.
(13) OSTERHOUT, W. J. V.
    1912. The permeability of protoplasm to ions and theory of antagonism. *In* Science, n. s., v. 35, no. 890, p. 112–115.
(14) ROBERTSON, T. B.
    1910. Über die Verbindungen der Proteine mit anorganischen Substanzen und ihre Bedeutung für die Lebensvorgänge. *In* Ergeb. Physiol., Jahrg. 10, p. 216–361. Literatur, p. 216–264.

Barley plants showing growth as affected by various salts in clay-adobe soil.

Fig. 1.—*A*, *B*, Growth with 0.2 per cent of sodium chlorid alone. *C*, *D*, Growth with 0.2 per cent of sodium chlorid and 0.2 per cent of sodium carbonate. *E*, Growth with 0.2 per cent of sodium carbonate alone.

Fig. 2.—*A*, *B*, Growth with 0.2 per cent of sodium chlorid alone. *C*, *D*, Growth with 0.2 per cent of sodium chlorid and 0.5 per cent of sodium sulphate. *E*, *F*, Growth with 0.2 per cent of sodium sulphate alone.

(218)

2 18

A   B   C   D   E

A   B   C   D   E   F

A NEW WHEAT THRIPS

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INTRODUCTION

A new wheat thrips, *Prosopothrips cognatus* Hood, was described by Mr. J. D. Hood,[1] of the Biological Survey, from material collected by the writer in 1908. As it is a new species without synonyms, there are, up to this time, no records of its having been destructive. The data contained in this paper have been collected during the last few years whenever the insect occurred in numbers sufficient for study. This species frequently becomes injurious to wheat (*Triticum* spp.) in localized areas, but has not yet been found doing injury to oats (*Avena sativa*) or other grain crops.

DISTRIBUTION OF THE SPECIES

This wheat thrips occurs in all parts of Kansas, even to the western border; in Oklahoma; at two places in western Missouri; and in one locality in extreme southern Nebraska. Careful search has been made for it in northern Texas, eastern New Mexico, and western Nebraska, and in Kentucky, Tennessee, and Georgia, with negative results.

DESCRIPTION AND LIFE HISTORY

THE EGG (PL. XXX, FIG. 1)

The female deposits her eggs in the tissue of the young leaves, usually on the ventral side, whether in wheat or grass, by first cutting the tissue with her sharp mandibles, then thrusting the short ovipositor into the lacerated leaf and placing a single, tiny egg in each puncture.

The egg when first deposited is translucent and nearly colorless, taking on a greenish tinge just before hatching. It is somewhat kidney-shaped, about 0.35 mm. in length, and 0.125 mm. at its greatest diameter.

The hatching period varies from 6 to 10 days and is about the same in the laboratory as under natural conditions in the field.

THE LARVA (PL. XXX, FIG. 2)

The greenish tinge observed a few hours previous to the issuing of the tiny, slender larva remains with the larva until after it begins to feed, when the color changes to a deeper green as the juices of the plant are imbibed.

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[1] Hood, J. D. Prosopothrips cognatus, a new North American Thysanopteron. *In* Canad. Ent., v. 46, no. 2, p. 57-59, fig. 13. 1914.

When newly hatched, the larva is from 0.39 to 0.40 mm. in length and from 0.09 to 0.10 mm. in width; when full grown it becomes about 1.0 to 1.2 mm. in length.   In general shape the body is fusiform, broadest at the middle of the abdomen, and tapering to a point at the anal end.   The antennæ are slender, their length being equal to one-sixth that of the body; the bases of the segments are shaded and set in proximal tubercles. There are no changes in the form during growth, except that the color becomes lighter during the last few days of the larval period.   The larvæ become full grown in from 10 to 12 days and crawl down the plants into the soil, where they pupate and transform to adults.   There is no indication of the yellow color of the adult when the larvæ enter the soil.   The pupal period lasts from 10 to 13 days, though no observations on this stage have been noted.

THE ADULT (PL. XXX, FIG. 3)

The adult, which was described by Hood,[1] may be readily recognized by the fact that it is a wingless form and has a pronounced yellow color, which distinguishes it from *Euthrips tritici* and other species of thrips commonly found on wheat.

NUMBER OF GENERATIONS ANNUALLY

The complete life cycle from egg to adult requires from 30 to 35 days. The egg hatches in from 6 to 10 days, the larval period occupies from 10 to 12 days, and the pupal period from 10 to 12 days, while the newly issued female requires but from two to three days to prepare for egg laying.   Although the length of life of an adult has not been definitely determined, a few have lived eight months in the laboratory.

The adults emerge from winter quarters as soon as the warm days of spring arrive, and the females soon begin depositing their eggs.   There are from four to five generations each year.   These overlap one another, so that adults and larvæ are present at all times, even in late winter. Larvæ are most numerous at heading time in the spring, also about the time volunteer plants come up in late summer, and again in late fall, when the wheat is getting a good start.   They continue to feed until the cold weather causes them to go into hibernation.

CROPS AFFECTED

Growing wheat is the only cereal that is known to have been damaged by this insect, although certain species of grasses are sometimes injured to a slight degree.   Wheat plants furnish its principal food from the time the volunteer plants sprout in August until the wheat crop is harvested the following June.   During the interval between wheat harvest and the sprouting of volunteer wheat the thrips feed and reproduce on *Agropyron*

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[1] Hood, J. D. Op. cit.

smithii, Elymus canadense, E. virginicus, Syntherisma sanguinalis, Panicum crus-galli, and *Hordeum jubatum.* They are found at all seasons of the year on these grasses, but more especially during the interval between harvest and wheat sowing.

INJURY TO PLANTS

The injury is confined to the leaves of young plants (Pl. XXX, fig. 4), unfolding heads and newly formed grains of wheat, and the young unfolding leaves of some grasses.

The method of feeding is similar to that of other allied species—that is, by first puncturing and lacerating the tissues of the upper epidermis of the leaf, or integument of the grain, then sucking out the juices. Both larvæ and adults feed in this manner, changing their point of attack frequently, and thus in a short time a leaf or grain is literally covered with lacerations.

The leaves when attacked by a dozen or more individuals at one time become badly mutilated in a few hours and, owing to the influence of sunshine and wind, soon acquire a "rusty" appearance. Since the injured leaves nearly always cover the next unfolding leaf, the injury often becomes disastrous to the plant by preventing the new shoot from developing. The heads are first attacked when in blossom, the pollen being eaten greedily. The tender stamens and pistils are lacerated badly and dry up very quickly, so that the embryo seeds are killed in a kind of injury seldom observed and one wherein the damage can hardly be estimated, although evidently it is considerable. As soon as the grains begin to form, the thrips attack the husk, and later, gaining access to the husk, they lacerate the tender integument of the newly forming grain. Grains attacked at this stage are practically destroyed, and even after the milk has become a dough the injury causes the grains to shrivel.

The last portion of a wheat plant to ripen is the head, and therefore the thrips remain on it until it becomes dry. They often stay on the green heads until harvest, but leave the plants very soon after these have been bound up into sheaves, afterwards subsisting on the common grasses present in the fields.

FIELD OBSERVATIONS

The depredations of this tiny insect were first brought to the writer's notice in the spring of 1908 at Pawnee, Okla., and Wellington, Kans. Here they were first observed in abundance, doing much damage to the new shoots of young growing wheat. With a few sweeps of the insect net they were collected by the thousands from wheat plants throughout April and May. In one instance where they were so very numerous the crop was not worth harvesting, but the failure of the crop could not be attributed entirely to the thrips, owing to the presence in abundance of

both the Hessian fly (*Mayetiola destructor* Say) and the chinch bug (*Blissus leucopterus* Say).

The thrips again appeared in the spring of 1909 in considerable numbers, but not enough to cause noticeable injury. In April and May many eggs and larvæ were killed by the hot sun and wind, on account of the drying of the wheat leaves. However, some of them thrived and reproduced freely, for in August, when volunteer plants sprouted, they occurred in large numbers and continued to reproduce and feed on the fall-sown crops until hibernation.

The winter of 1909–10 was very severe, and only a small number of this species survived; consequently, but few were found during the year 1910 and a still smaller number were noted in 1911 and 1912.

In the late fall of 1912 females were found in clumps of *Agropyron smithii* and also a number were in stools of wheat, in which they hibernated. Thrips were common on wheat and grasses during the growing season of 1913, hibernating in the late fall and appearing in swarms on young wheat in early March, 1914. By the 1st of April the larvæ, now nearly grown, were cutting the shoots severely. They ceased feeding about the third week of April and pupated. By the time the adults were ready to issue, the wheat plants had outgrown all previous injury. Favorable rains produced a rapid growth of wheat, which apparently did not interfere with oviposition or with the feeding of the young thrips larvæ. By the middle of May, when the wheat was heading, the second brood of larvæ readily infested the young heads, feeding upon the stamens, pollen, and pistils, and later attacking the integument of the grain. Larvæ and adult thrips continued to feed on the heads until the crop was harvested, very few being dislodged by the binder. Subsequently they clung to the heads for three or four days, finally leaving the grain for grasses or entering the soil for pupation.

Neither adults nor larvæ could be found in stubble fields during the summer, but as soon as volunteer wheat plants pushed up in early September the thrips were found in all parts of the field, which would indicate that they had been present all the time. As no evidence of thrips was found in the fields during the summer, it follows that the pupæ had waited for the rains, the moisture being sufficient to sprout wheat grains and also to cause the adults to issue.

The thrips did no appreciable injury to young plants in the fall of 1914, but in early December of that year many were hibernating in the principal host plants, the stools of wheat and clumps of *Agropyron smithii*.

HIBERNATION

The adults and larvæ hibernated in clumps of wheat, *Andropogon scoparius*, and other grasses in the fall of 1909. Although living adults and larvæ were found in wheat on February 8, 1910, and again on March 1,

indicating that they had successfully passed the winter as both adults and larvæ, no eggs could be found on or in wheat leaves.

Both adults and larvæ have been found hibernating beneath the sheaths of the following grasses: *Triticum vulgare* (wheat), *Andropogon scoparius*, *A. furcatus*, *Poa pratensis*, thick mats of *Agropyron smithii*, and *Tripsacum dactyloides*. No stages of thrips have been found hibernating in *Panicum crus-galli* or *Syntherisma sanguinalis*, which become quite dead and dry after the first frost and are abandoned, the thrips continuing to feed on other plants during the warm days that usually follow.

ENEMIES

Among the more important enemies of *Prosopothrips cognatus* are *Triphleps insidiosus* Say and the larvæ of *Chrysopa oculata* Fab., which consume large numbers. When the thrips are numerous, the fields are literally swarming with the Triphleps. No coccinellid adults or larvæ, not even of the smaller species, have been observed feeding on them, either in the field or in confinement. No parasites have been reared, although it is possible that some parasite materially assisted in reducing their numbers in 1910. No birds have been observed feeding on them.

CONTROL

At this time no thoroughly practical remedy can be offered for the control of this pest. Large numbers of thrips may be destroyed by burning off all grasses, but the young wheat fields, where most of the pests are located, can not, for obvious reasons, be burned; nor is it practicable to spray wheat since the expense of the operation would be greater than the returns.

Careful observations on plowing at different times from the middle of June until September seem to favor early plowing, for, although all fields under observation were infested, the two plowed in June and harrowed in late July were attacked to a lesser degree, and these fields contained practically no volunteer wheat.

Where this species becomes numerous, it appears that when stubble fields are burned over and plowed early, destroying all grasses, and especially volunteer wheat, there is less opportunity for the thrips to increase in numbers sufficient to damage the crop.

PLATE XXX

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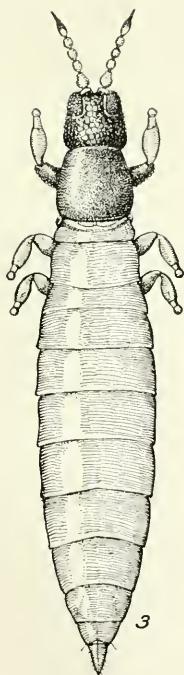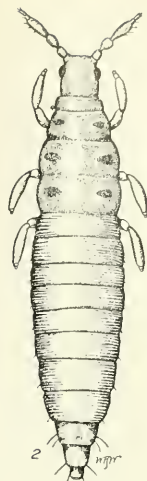
CYTOLOGICAL STUDIES OF AZOTOBACTER CHROOCOCCUM [1]

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INTRODUCTION

Because of its advantage to agriculture, the maintenance of a nitrogen balance in the soil has long been studied by scientists. Among the lower organisms involved in this phase of agricultural economy the Azotobacter group, first described by Beijerinck (2)[2] in 1901, is surely one of those that deserve very close study because of their extreme importance.

Since the first work of Berthelot (4) on nitrogen fixation by soil, the Clostridium and Azotobacter groups have been discovered and the methods for their study have been brought to the standard basis, so that these organisms can now be justly compared to any other organism belonging to the class of the Protista. Unfortunately, not till very recently has the cytology of *Azotobacter chroococcum* been studied. But since cytology in all the applications of bacteriology, such as pathological bacteriology and the bacteriology of the tanning, retting, and fermentation industries, has been overlooked by many, it is not surprising that such studies have been deemed unnecessary or unimportant in agriculture.

It is a rational hypothesis very often expressed that every change in physiological activities is accompanied by a like change in the cytological structure of an organism.

Unfortunately, with our present methods, there is no possibility of studying changes in structure, especially if these are small, with that accuracy which it is possible for us to employ in detecting changes in physiological activities.

Furthermore, the majority of methods commonly used in bacteriological studies, especially the staining methods and processes, are only of a diagnostic and medical value, unsuited for refined work. More delicate methods should be used if very slight differences in structure are to be detected.

Although the size of *A. chroococcum* makes the organism ideal for a cytological study, it has been completely ignored for years. Its biological functions play such a great rôle in the nitrogen cycle in nature

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[1] Thanks are due to Dr. E. R. Allen, under whose direction this work was undertaken, for kind advice and criticism.

[2] Reference is made by number to "Literature cited," p. 238-239.

under experimental conditions that the question may well be raised whether such functions are equally performed under what might justly be termed "abnormal conditions."

It is ordinarily assumed that the natural condition for *A. chroococcum* is the one in which the organism is obliged to fix nitrogen, since the name "nitrogen fixer" is applied to it. We know now, as a result of considerable work on the part of a number of scientists, that the growth of *A. chroococcum* is stimulated by the presence of small quantities of nitrates, and we know, moreover, that *A. chroococcum* has the power, according to Beijerinck and Van Delden (3), to transform nitrates directly into ammonia, and that, according to Sackett (16, p. 38–39), it forms more of the characteristic pigmentation when grown in a medium containing 0.5 per cent of sodium nitrate than in a medium poor in, or free from, nitrates.

We know also from Sackett (16, pp. 38–39) that ammonium chlorid, ammonium sulphate, asparagin, and peptone do not have the same effect upon this function. The quantity of sodium nitrate used by this author is five times stronger than the one used in ordinary denitrification experiments.

Since this work indicates that a highly oxidized nitrogenous material, such as a nitrate or nitrite, is the only one that seems to accentuate growth and that this same material is attacked and consumed by *A. chroococcum*, are we not justified in believing that the condition of life presenting these compounds as foods might constitute a favorable and perhaps an optimum or normal condition for development? In other words, *A. chroococcum* may be a nitrogen fixer under only such conditions as those which we call "normal"—i. e., when a lack of soluble nitrogenous food is present—and a denitrifier when such conditions are changed—i. e., when there is a possibility for it to consume nitrate under "naturally normal" conditions. It is well known that this micro-organism is a facultative nitrogen fixer, but which is its normal and which its abnormal function is as yet an unsolved problem. A cytological study might possibly throw some light on the question. Appreciable changes in structure, detectable by appropriate methods, might accompany changes in physiological functions. An example of this kind of change is given in the recent work by Nello Mori (13), in which he finds that the cultivation of *Caryobacterium equi* (a pathologic form) on carbohydrate or alcoholic media individualizes in the cells of the organism a so-called nucleus, which was not visible in cells grown on peptone medium.

A study of the change due to environment in a unicellular organism will quite naturally simplify a corresponding study in higher plants, in which there have been so much dissension and contradiction. Before undertaking this study we should determine the nature of the cell constituents which are capable of undergoing changes with variation in

environmental conditions. With the object in mind of clearing up this point, a brief review of the literature on the cytology of *A. chroococcum* is submitted.

HISTORICAL REVIEW

Beijerinck (2) early described *Azotobacter chroococcum* with regard to its morphology. With the aid of Prof. Zetnow, he determined that the organism was capable of movement by means of a polar cilium. This author describes sarcina-like packets in cultures of *A. chroococcum* and also points to the supposition that these might be resistant forms to replace the spore stadium, which is not present in this organism. These same packets were observed by Krzemieniewski (10, p. 932–941), who found, furthermore, that the cells escaped later from their envelope, leaving it like an empty sheath. Their resistant nature was to some extent doubtful.

A very interesting contribution to the cytology of *A. chroococcum* was made by Prazmowsky (15), who used nearly exclusively vital staining with methylene blue to determine the structure of this organism. He observed the division of what he called "nuclei" (which, according to the description, are Heinze's (8, p. 57) glycogen granules, Ashby's (1) glycogen granules, H. Fischer's (5) volutin granules, and Mencl's (11) nuclei) in the cells of this Azotobacter and stated that the division of this nucleus was followed by the division of the entire cell. By this means were formed chains of elements, which later separated in single individual cells. Prazmowsky denies completely the presence of glycogen in vegetative cells of *A. chroococcum*, but admits its presence in the cells representing the resting stage of the organism—the so-called arthrospores. He holds to the presence in nature of three types of cells of *A. chroococcum:* (1) Nuclear cells (*Kernzellen*), (2) alveolar cells (*alveoläre Kernzellen*), and (3) cells with a diffused nucleus (diffuse *Kernzellen*). Some drawings presented by Prazmowsky (15) resemble the sarcina-like packets described by Beijerinck (2) and pictured by Krzemieniewski (10).

Jones (9) holds views completely different from those of all the preceding authors. He considers the granules within the cells of *A. chroococcum* to be of two kinds, one kind, more often found, consisting of glycogen, and the other of a substance that makes up the body of what the author calls "gonidia," which are capable of flowing from the mother cell and are provided with very long delicate cilia.

Mencl, in his work already mentioned (11), used the same staining methods as Prazmowsky (15), and came to the same conclusions as that author.

From this very brief summary of the literature on the subject it is evident that opinions as to the constitution of the cellular make-up of *A. chroococcum* differ widely.

Since the most important constituents of the cell seem to be the granules, a study of their constitution will be first taken up. So many different hypotheses have been presented with regard to their constitution that the following studies were undertaken in order to determine it.

EXPERIMENTS WITH THE ORGANISM

CULTURE USED

After having observed the presence of granules in the cells of some cultures of *A. chroococcum*, microchemical studies were undertaken to determine their nature and to ascertain whether they could be stained in such a way as to be easily individualized in future studies. Some microscopical preparations had already shown that the Guignard (6, p. 19) method was the one to be depended upon, but to study this point better, a series of slides were prepared and stained from an old stock culture that had been cultivated on ordinary mannit agar for two years without having at any time been rejuvenated in soil. The culture had apparently lost the power to produce pigment, as it had not produced any for the last five or six transfers on solid medium. This culture was used in all the experiments described in this paper.

The growth on mannit agar was white, transparent, strongly raised at the beginning, flattening with age, smooth, soft, and invading the slant at the bottom.

The culture had been forwarded to the Ohio Station by the Office of Soil Bacteriology and Plant Nutrition, United States Department of Agriculture, and had been obtained by that institution from the American Museum of Natural History. At the time it was received in this laboratory it bore the label "*Azotobacter chroococcum* Beij." and has been kept ever since in pure culture.

When cultivated on mannit agar, to which had been added 1 gm. of potassium nitrate per liter, the culture regained its pigment-producing power, assuming a waxy and glossy appearance.

Since the organisms vary from cocci forms to bacillary ones, no single measure can be given to the species, but a distinction must be drawn between these two forms. The cocci forms measured 1 to 2μ in diameter and the bacillary forms 3 to 4μ by 1.5 to 2μ. The nitrogen-fixing power of the organism was very slight, since it fixed only 1.26 mg. of nitrogen in 25 c. c. of solution in 37 days in pure culture.

STAINING THE ORGANISM

Many methods of staining the organism were tried in the hope of finding one that would be adapted to follow up the growth and rôle of the granules in the metabolism of the cell. All the methods used gave satisfactory results. The solutions were tested several times on blood as a

standard stainable substance. Since the nature of the components of polymorphonuclear leucocytes is known and since the solutions responded alike on these and on the cells of *A. chroococcum*, we are justified in comparing these bacterial cells with the blood cells.

GUIGNARD'S METHOD was originated by A. Guignard (6, p. 19), who used it to study the cytology of antherozoids. The organism was fixed with osmic-acid fumes and stained in a mixture of 50 c. c. of a solution of 2 per cent of fuchsin in 1 per cent of acetic acid, 40 c. c. of a solution of 0.2 per cent of methyl green in 1 per cent of acetic acid, and 1 c. c. of acetic acid. This method gave a splendid picture of the structure of the cell. The network, of which mention will be made later on, was stained violet, the contents of the network were hardly stained at all, and the granules were a deep violet-black.

The Guignard (6, p. 19) method did not give the color differentiations desired, red and green, perhaps on account of metachromacy of the methyl green used in the solution. On the whole, it proved to be a very satisfactory method. Plate XXXI, figures 2, 3, 4, 6, 8, and Plate XXXII show some very good preparations obtained by it; the network showed plainly and the granulations very distinctly. Plate XXXIII, figure 1, shows some cells stained by this method; several granules are to be seen. The cell wall is quite evident in old cells, but in young ones it is to be seen only slightly stained.

HEIDENHAIN'S ORDINARY FERRIC HÆMATOXYLIN stained the network strongly, but gave no differentiation. The fixing was done by passing the glass through a flame.

The Heidenhain method was used progressively in a great number of cases and regressively only occasionally. When progressive, it stained the cell components black, and it demonstrated the zooglea sheaths, such as Beijerinck (2) and Krzemieniewski (10) found. Plate XXXI, figure 5, shows some cells obtained by the Heidenhain method. Plate XXXIII, figure 3, shows some zooglea and cells escaping from them, and Plate XXXIII, figure 2, shows two of these zooglea nearly empty, all cells having escaped, the same as is shown in Plate XXXI, figure 5. In some groups the walls which formerly separated the different cells in the same mass are to be seen quite distinctly after the cells have escaped (Pl. XXXI, fig. 5).

Smears from a hay-agar culture of *A. chroococcum* stained by the regressive method showed the network to be made up in many cases of a substance taking a beautiful dark-violet color and of a nearly transparent appearance.

ROMANOWSKY'S SIMPLE STAIN is the one used by the originator for the study of the parasitology of malaria. The preparation was fixed by the flame and stained in a mixture of five volumes of eosin in a 1 per cent aqueous solution and two volumes of a saturated aqueous solution of

methylene blue. This method gave nearly as good results as Guignard's (6, p. 19), but no color differentiation was obtained.

The Romanowsky simple method was not the most satisfactory, on account of the lack of color differentiation, although it demonstrated quite clearly the cell structures.

METHYLENE BLUE, 1 to 1,000, in aqueous solution (flame-fixed), gave good results; but, although repeated examinations were made of the slides thus prepared, no red color was visible, the granules being indicated only by a strong black-blue color.

ROMANOWSKY'S COMPOUND STAIN (after Harris). No fixing was done, since the methyl alcohol itself served as a fixing agent. Staining was done by the mixture of methylene azure and methylene violet and with eosin and methylene blue in methyl alcohol. This method, although very good in showing the structure of the cells, failed in most cases to give sharp differentiation. No real red color for chromatin was obtained, as the one that would result from the staining of the hæmoparasitic protozoa. Some human blood stained by this solution gave the usual color presentations. Leucocytes of all kinds, polymorphonuclear, macronuclear, and eosinophylic, were seen, stained in their characteristic and distinct colors. When *A. chroococcum* was stained by this method, the network took a blue color and the interior of the meshes a pink one. Repeated trials with the methylene-blue and glycerin method as used by Mencl (11) failed to give satisfactory results; the cell always took the blue, and the granules never took the red color that Mencl ascribes to them. Vital stained cells present the same structure as the one described by Mencl (Pl. XXXI, fig. 1).

In the Romanowsky-compound method great attention must be paid in diluting on the cover glass, since the solution tends to flow on the underside and make a miscroscopical examination difficult. The time of action for maximum differentiation varies with different objects. After several trials the time limit for the action of the stain in these tests was fixed at 10 minutes. After the addition of water to the stain on the cover glass, the washing should be very rapid. The best preparations were obtained when the washing was not prolonged over half a minute. Distilled water was preferred in the washing.

The cells of *A. chroococcum* stained differentially; the network took on a blue color, while the contents of the network meshes took on a pink color. If the washing is prolonged, the pink color disappears and only the blue remains in the cell, as in blood stained by this method.

MICROCHEMICAL STUDIES

Since the work of Mencl (11), Jones (9), Prazmowsky (15), and others has shown that the structure of the cell of *A. chroococcum* is complex, the present writer next proceeded to determine microchemically the nature of the different components.

The methods used are those proposed by A. Meyer (12) and by his follower Grimme, and are already recognized as valuable in the study of the cytology of bacteria.

The granules observed by the writer in the cells of this organism might be nuclei, metachromatic granules, fat drops, glycogen, or starch.

Mencl (11) and Prazmowsky (15) believe them to be nuclei or nucleo equivalents; Jones (9), glycogen granules and nuclei of gonidia; and Fischer (5), metachromatic bodies. None of these authors has attempted to prove his point satisfactorily or to disprove other possibilities.

Since the aim of this part of the work is to find the true nature of the granules met with in the organism in question, the methods used will be described and the results of the investigations given.

STARCH.—Smears from cultures on mannit agar, flame-fixed, were mounted in a saturated aqueous solution of iodin, sealed with paraffin, and observed at once and 24 hours later.

The granules are not to be considered as starch, since they do not give a blue color to iodin or to Meissner's solution.[1]

Even after 24 hours no coloration was visible. The cell diaphanized, and the granules gained in refraction.

FAT.—Smears from a mannit-agar culture were immersed in ethylic ether for $1\frac{1}{4}$ hours, dried, stained in methylene blue, and mounted in balsam.

Since A. Meyer (12) claims that chloroform and alcohol are not good solvents of bacterial fats, on account of the difficulty which they meet in passing through the cell wall and *Schleimschicht*, the writer tried to avoid any objection that could be raised against the conclusions drawn from the results obtained by the ethylic-ether method.

With this aim in view tests were made, using the method suggested and recommended by Meyer. It consists in fixing the bacterial smear in formol, immersing in glacial acetic acid, neutralizing, and staining. Methylene blue, 1 to 10, was used as the stain; it gave very good preparations. The fats are dissolved by this method. Treatment with glacial acetic acid did not dissolve the granules.

Prolonged treatment in the cold with ether or with glacial acetic acid did not dissolve the granules, since those which had been treated stained just as strongly as the checks. This eliminates the question of their fatty nature, although their deeply staining property should have already led to this supposition. It should be noted that these preparations were not fixed in osmic acid, which treatment would render lipoids, or fatty substances in general, insoluble in fat solvents, and stainable by the ordinary staining solutions. For this reason their myelin nature should not be accepted.

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[1] Formula for Meissner's solution: Metallic iodin, 7 gm.; potassium iodid, 20 gm.; water, 100 gm.

GLYCOGEN.—Smears were mounted in a saturated aqueous solution of iodin, sealed with paraffin, and observed at once or 24 hours later.

Meissner's solution instead of iodin was also used.

A. Meyer (12) also suggests boiling for three minutes in a weak solution of sulphuric acid (2 drops of concentrated H_2SO_4 in 5 c. c. of H_2O). Glycogen is dissolved by this treatment.

The granules did not give any golden color with iodin solution, even after 24 hours, contact. Meissner's solution, not removed from cells, gave a very dark golden-yellow color. To be certain, however, of the presence of glycogen in the cells, this golden color should persist after the excess of Meissner's solution has been replaced by water by means of capillarity. The preparations of the writer, nevertheless, did not retain the golden coloration after capillary washing of the mount, although a strip of filter paper placed on the edge of the cover glass in contact with the wash solution took on a faint-blue color.

The granules were dissolved by the treatment with sulphuric acid. It is to be remembered that also metachromatin is dissolved by boiling in water for three to five minutes. If the sulphuric acid in the cold were not to dissolve the latter, perhaps it would at the boiling temperature. Moreover, the sulphuric-acid solution used for testing the solubility of metachromatin is 1.2 to 1.3 times stronger than the one used for the detection of glycogen by this method, which would probably account for the solution of the granules. The granules in this case, according to my view, are not of a glycogenous nature.

The mounting of smears in a dilute Meissner solution (2 drops in 5 c. c. of H_2O) showed the cells stained in a homogenous manner straw-yellow, while the mounting of a smear of a blastomycete (*Saccharomyces cerevitiae*) in the same solution gave a decided golden-brown granulation.

METACHROMATIC AND CHROMATIC GRANULES.—To distinguish between the two kinds of granules, several tests were used.

(*a*) The ruby-red color, which should be developed by the Romanowsky-compound method, indicates chromatin. Protozoa are a good example of the results to be obtained.

In the large number of slides prepared no ruby color was developed by the Romanowsky stain.

(*b*) A cover-glass preparation was stained with methylene blue, 1 to 11. After washing in water treated with 1 per cent of sulphuric acid, chromatin should discolor at once, while metachromatin should not.

Treatment with the sulphuric-acid solution did not decolorize the granules, but it decolorized the cell network.

(*c*) A cover-glass preparation stained with methylene blue was treated with a 5 per cent solution of sodium carbonate. Chromatin should remain colored; metachromatin should discolor.

Treatment with the sodium-carbonate solution discolors the granules, leaving the cell network unchanged.

(*d*) A cover-glass preparation stained with methylene blue was treated with Meissner's solution and then with a 5 per cent solution of sodium carbonate. After treatment with the Meissner solution the cell should be brown and the granules decolorize if of metachromatic nature; they should remain colored if of chromatic nature.

This treatment left the granules decolorized and cells stained blue.

(*e*) On treating with boiling water and then staining or boiling in a test tube for two minutes and then staining, the metachromatin should dissolve.

Boiling with water over Bunsen burner for two minutes completely dissolved the granules. Washing with water at 90° to 99° C. for four minutes did not dissolve all the granules, perhaps on account of insufficient temperature; thus, only some granules, the large ones much reduced in size, are to be seen after this treatment. The contents of the network took a light-pink color when treated with methylene blue, 1 to 11.

(*f*) Smears were prepared and stained by the Ernst method. Blue coloration of granules after the Bismarck-brown treatment would therefore indicate metachromatin.

This method gave a bluish color to the granules, but these preparations do not last when mounted in balsam (dissolved in chloroform).

(*g*) On staining unfixed preparations with methyl green the dye should stain only chromatin.

Staining with methyl green showed a strong metachromacy, since the granules took on a violet color. The staining of unfixed preparations in solutions of Grubler's methyl green gave a violet color to the granules. Later, in trying to explain this metachromacy, tests were made on the purity of the stain. They showed it to contain considerable impurities in the form of violet dyes, probably methyl violet.

Mounts in 5.N potassium hydroxid and in calcium chlorid gave preparations that did not especially change in appearance from the checks.

RESULTS OF MICROCHEMICAL TESTS

From what has been said about the granules found in the cells it may be stated that their nature seems to be different from that of the granules found by Mencl (11), Jones (9), Prazmowsky (15), and others in the organism on which they were working, with the exception of those treated by Fischer (5).

In fact, as has been seen, the granules found by the writer did not give the reaction for glycogen. The results obtained with Meissner's solution must be carefully judged before making a definite statement. If the granules were glycogenic, they would not only give a golden color when treated with the reagent but would also retain it if the excess of the

reagent were extracted, just as filter paper would if treated with the same solution and then an excess of the reagent washed off.

The color might disappear if the washing were very prolonged, but in this writer's case the washing was not thorough, because when the cover glass was sealed to the slide a strip of white filter paper, brought in contact with the solution, gave a bluish color, indicating presence of a reagent· Moreover, as Meyer (12) has shown, a very small amount of a reagent is necessary to give reliable results. Besides, treatment with a concentrated solution of iodin for 24 hours gave no trace of coloring whatever to the granules. Treatment with Meissner's concentrated solution of cells of the organism, kindly forwarded us by Prof. D. H. Jones, gave a strong golden-brown color.[1]

It should be observed that the trials were repeated also on old cultures (14 or 15 days old), but no reaction took place. This would be contrary to the statement by Jones (9) to the effect that the cells give less glycogen reaction when young than when old. Also in one single slide of a comparatively young culture there ought to be many fully developed cells that should react. It therefore appears natural that the granules observed by the writer should be classed among those which, according to Jones (9), "do not always appear to be present, do not give the glycogen reaction, but do stain with various aniline dyes."[2] Their failure to take the blue color with iodin excludes their starchy nature. They are not fats, because treatment with ether did not dissolve them, because they stained with extraordinary facility, and also because they were not dissolved by treatment with glacial acetic acid. It is very probable that the granules observed by Mencl (11), which stained easily with methylene blue, are the same as those of Jones (9), just quoted.

Although repeated trials were made to obtain the color differentiation reported by Mencl (11) with methylene blue and glycerin, the results were always unsatisfactory. The granules never stained red.

Mencl (11) and Prazmowsky (15) class the granules as "nuclei- or nucleo-equivalents," thus implying that they have a chromatic nature. Jones (9) considers them as motile flagellated granules, resembling the reproductive organs of many Cyanophyceae. This naturally implies that they contain chromatin as a necessary constituent. Neither of these authors proved his assertions with standard methods.

The granules that were obtained by the writer stained easily and deeply, but gave no reddish color with a 1 to 1,000 methylene-blue solution, though they gave it with a 1 to 5,000 solution, as metachromatic granules usually do. They responded positively to all the tests carried out to distinguish their metachromatic character.

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[1] Since this method is not reliable, the present writer intends, in due time, to make a quantitative determination of the glycogen to be found in large quantities of cells of *A. chroococcum* grown under conditions equal to the preceding.

[2] As to the ciliated gonidia described by Jones (9), see under "Staining the organism" and "Discussion of results."

A summary of the results of the tests to distinguish between meta-chromatic and chromatic granules is given in Table I.

TABLE I.—*Summary of results of the tests for metachromatic and chromatic granules* [a]

Treatment.	Theoret-ical results: metachro-matin.	Actual ex-perimen-tal results: granules.
(b) Methylene blue (1:10)[b] and 1 per cent of sulphuric acid	+	+
(c) Methylene blue (1:10)[b] and 5 per cent of sodium carbonate....	−	−
(d) Methylene blue (1:10),[b] Meissner's solution, and 5 per cent of sodium carbonate	−	−
(e) Boiling for 2 minutes in water	−	−
(f) Ernst method, blue color for metachromatin ("volutin")	+	+
Weak methylene-blue solution shows red color	+	+

[a] + signifies a coloration; −, a discoloration.
[b] Methylene blue (1:10)= 1 part of methylene-blue saturated solution added to 10 parts of water.

As can be seen from Table I, there is no doubt that metachromatic or, as A. Meyer (12) terms them, "volutin" granules were found.

DISCUSSION OF RESULTS OF THE EXPERIMENTS

Since, according to the tests performed, the metachromatic nature of the granules is assured, their action in the cell will be studied to ascertain whether they have a rôle in the metabolism which confirms the microchemical investigations.

As we know, Meyer (12) considered metachromatic granules as reserve foods, while MacCallum and Carlier, according to Guilliermond (7, p. 199), considered them as "zymogenic granules." Although the writer does not believe a conclusion as to whether the granules of the writer are reserves or zymogens can be reached in the present paper, he thinks that by presenting some points of the question a working hypothesis may be sketched for future work.

One of the main points for consideration is the disposition of these granules in the cell—i. e., to determine whether a regular disposition takes place at some time in the life of the cell or whether the granules have no special setting in the organized unit.

To begin with, the drawings furnished by Mencl (11) will be studied. In nearly all cases in which a reticulated structure is to be seen, the granules are placed on the juncture of the net meshes or also in the center of the meshes themselves. As to the regularity of distribution in the cell, these granules present none, because, as Mencl himself states, all stadia, from fine scattered points to large globules, are to be found. Considering the large globules as the maximum development of the material composing them, the most advanced stage morphologically, they should be expected to occupy a uniform place in the fully developed

cell.   From Prazmowsky's work (15) nothing can be decided with regard to this point.   This author does not give much importance to it, and his drawings show the same thing as those of Mencl.

Might not a regularity in the setting of the granules, not determined by the above-named authors because of unappropriate staining methods, be presented at some time during the life of the cell?

To determine this point it is necessary to know just what are the changes which the cells of *A. chroococcum* undergo with age.   These queries will have to be solved in the following order:

(I) What are the changes in the cytology of *A. chroococcum* at different ages of the same cell?

(II) What is the relation of the changes in cytology to the fate of the granules of the cell?

Since difficulties in operative technique make the solution of Question I impossible,[1] its wording must be changed to the following: (I) What are the cytological changes undergone with age by the cells of *A. chroococcum?*   Plate XXXI, figures 6 and 7, gives a graphical solution to this question.   A few words must be said about the stadia which were found in the life cycle of the organized units.

That the reticulated structure found in the cells in the writer's tests was not due to the drying before fixing or to the fixing is proved by the fact that many observations of vital-stained preparations made in this laboratory and also by other authors showed the same structures as did the treated cells.   Moreover, the different methods of fixing used, osmic-acid fumes, methyl alcohol, and flame—i. e., gas, liquid, and heat—all gave the same structures.   Therefore these structures can not in any degree whatsoever be considered as artifacts produced by the fixing agents.

The first stadia were found with undifferentiated cytoplasm and metachromatic granules (Pl. XXXI, fig. 6, No. 1; fig. 7, No. 1, 2, 3).

Second, a stadium of cytoplasmic differentiation or reticulation was found in which the cytoplasma contracts toward the sides of the cell, leaving some strands to connect the accumulations of cytoplasma placed in different places of the periphery (Pl. XXXI, fig. 6, No. 2–10; fig. 7, No. 4–7).

Third, a division stage was found, in which the cells after elongation and differentiation in peripherical and transversal cytoplasma divide and form a wall between the resulting daughter cells.   The second and last stadia are interdependent.

Such a succession of stadia would then be in conformity with the one resulting from the studies on *Bacillus anthracis* by Pénau (14).   The nuclear phase was not found in the organisms in the experiments of the

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[1] Vital staining, although very valuable in following cell division, could hardly give here results to answer the question.   Prazmowsky's (15) and Mencl's (11) interpretations show the disadvantages of the method.

present writer; perhaps because he did not use the same methods as Pénau. As can be seen from the accompanying illustration (Pl. XXXI, fig. 6, 7) the granules do not disappear from the cells at any time of the life cycle of the latter; neither do they show any uniform setting. Possibly they would correspond in *A. chroococcum*, to the so-called nuclei of *B. anthracis* if they did not exist already in the undifferentiated stadium and if they did not persist in the reticulated stadium. As Pénau states, the metachromatic granules already exist in the undifferentiated cells of *B. anthracis* and persist in the reticulated cells, while the structure which he calls true nucleus disappears at the outset.

In Plate XXXI, figure 6, No. 6, and in Plate XXXII, it can be seen that the granules may even be placed on the outside of the cell. Mencl (11) also has noted this fact, but does not attempt to give any explanation in regard to it. The granules increase in size with age, but, aside from this character, they present no other.

Their plurality could not by itself exclude their nuclear nature, but that character in addition to their occasional extracellular position would be sufficient to deny to them nuclear functions. This point was not considered by Mencl as one worthy of attention.

How could he consider these granules, escaping from the cells as nuclei, as the most important and most vital organs of a cell? It is true that in higher plants some cells (sieve tubes) are also left in old age without nuclei, but it is also true that in most cases these cells are no longer capable of reproduction. These granules have also been found to escape from cells which were undergoing the process of division. No isolated mass of cytoplasm has ever been seen to divide spontaneously.

Plate XXXI, figures 8 and 8a, shows some other cells stained by the Guignard (6) method. All stages are here represented.

Some cells, which are easily found, do not contain any granules, although their size indicates an advanced age. This lack of granules might possibly be attributed to an expulsion of the same by the method just mentioned.

To judge from Mencl's (11) drawings, many cells present one granule, but no regularity as to its setting in them. In Plate XXXII, which represents some cells drawn at random from a Guignard-stained preparation, the granules are always seen embedded in the cytoplasmic matrix [1] and are never to be seen inside the meshes of the cell network. Sometimes

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[1] The expressions "cytoplasmic matrix," "cell network," "cytoplasmic strands" are here used to mean that part of the cell contents that in our organisms has an affinity for basic dyes.

As we have already seen, the Romanowsky compound stain gives a differentiation of colors in the cells, the network taking the blue color characteristic of nuclei and the contents of the meshes taking a pink color. Some comparative preparations with normal and abnormal human blood were studied; the same staining solution as the one used for *A. chroococcum* gave the ordinary colors.

Nothing should now prevent the naming of the basophylic cell constituents "nucleo substances," and the eosinophylic ones "cytoplasm." But since we mean to furnish more proofs to establish their nature, we will continue to use the terms "matrix," "network," "strands," according to their morphology. See also Plate XXXI, figures 2, 3, and others.

at first glance they seem to be completely detached from the cytoplasmic network, but a closer observation shows very feebly staining joining strands. Preparations from liquid cultures also present the same cellular structures.

CONCLUSIONS

From the present paper the following conclusions may be drawn:

(1) The cells of *Azotobacter chroococcum* Beij. present a complex nature and different stadia of cytological make-up. Conforming to the conclusions of Pénau (14) on *B. anthracis* and all endosporous bacteria, *A. chroococcum* shows an undifferentiated stadium. The nuclear stadium and the sporogenous one were not studied in the present paper.

(2) The organism with which we are working presents peculiar granulations, which seem not to have any relation to the reproduction of the cell.

(3) These granulations take the basic dyes and are constituted neither of fats nor glycogen, starch nor chromatin. They seem to be of a metachromatic nature.

(4) They seem to have their genesis from the nucleus, since they are always to be found embedded in that part of the protoplasm which shows nuclear characteristics.

(5) Their disposition in the cells is not regular, but changes in different individuals.

(6) Their place in Meyer's (12) system is uncertain, since by the present work on their nature they seem to belong to the class of ergastic structures, or stored material, while according to Prazmowsky's (15) work their reproduction might place them in the class which Meyer calls "protoplastic." Their regular appearance in the cells of *A. chroococcum* might be caused by the special conditions of life.

LITERATURE CITED

(1) Ashby, S. F.
  1907. Some observations on the assimilation of atmospheric nitrogen by a free living soil organism.—Azotobacter chroococcum of Beijerinck. *In* Jour. Agr. Sci., v. 2, pt. 1, p. 35–51.
(2) Beijerinck, M. W.
  1901. Ueber oligonitrophile Mikroben. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 7, No. 16, p. 561–582, 1 pl.
(3) ———— and Delden, A. van.
  1902. Ueber die Assimilation des freien Stickstoffs durch Bakterien. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 9, No. 1/2, p. 3–43.
(4) Berthelot, M. P. E.
  1885. Fixation directe de l'azote atmosphérique libre par certains terrains argileux. *In* Compt. Rend. Acad. Sci. [Paris], t. 101, no. 17, p. 775–784.
(5) Fischer, Hugo.
  1906. Zweiter Beitrag zur Kenntnis der Lebensbindungen von Stickstoff sammelnden Bakterien. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 15, No. 7/8, p. 235–236.

(6) GUIGNARD, Léon.
    1889. Développement et constitution des anthérozoïdes. *In* Rev. Gén. Bot.,
        t. 1, p. 11–27, 63–78, 136–145, 175–186, pl. 2–6.
(7) GUILLIERMOND, A.
    1906. Les corpuscles metachromatiques ou grains de volutine. *In* Bul. Inst.
        Pasteur, t. 4, no. 4, p. 145–151, fig. 1–4; no. 5, p. 193–200, fig. 5–8.
(8) HEINZE, Berthold.
    1904. Ueber die Bildung und Wiederverarbeitung von Glykogen durch niedere
        pflanzliche Organismen. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 12,
        No. 1/3, p. 43–78; No. 6/8, p. 177–191; No. 11/16, p. 355–371.
(9) JONES, D. H.
    1913. A morphological and cultural study of some Azotobacter. *In* Centbl.
        Bakt. [etc.], Abt. 2, Bd. 38, No. 1/6, p. 14–25, pl. 1–5.
(10) KRZEMIENIEWSKI, Severin.
    1908. Studya nad Azotobakterem.—Untersuchungen über Azotobacter chroo-
        coccum Beij. *In* Bul. Internat. Acad. Sci. Cracovie, Cl. Sci. Math. et
        Nat., 1908, no. 9, p. 929–1051, pl. 31.
(11) MENCL, Emanuel.
    1911. Die Kernäquivalente und Kerne bei Azotobacter chroococcum und
        seine Sporenbildung. *In* Arch. Protistenk., Bd. 22, Heft 1, p. 1–18,
        pl. 1.
(12) MEYER, Arthur.
    1912. Die Zelle der Bakterien. 285 p., 34 fig., 1 col. pl. Jena.
(13) MORI, Nello.
    1913. Di un nuovo batterio patogeno e di molti altri batteri nei quali può
        provocarsi l'individuazione di un nucleo tipico. *In* Ann. Staz.
        Sper. Mal. Infet. Bestiame [Napoli], v. 1, 1911/12/13, p. 265–322, 1 pl.
(14) PÉNAU, Henry.
    1911. Cytologie de Bacillus anthracis. *In* Compt. Rend. Acad. Sci. [Paris],
        t. 152, no. 10, p. 617–619.
(15) PRAZMOWSKY, A.
    1912. Studya nad Azotobakterem. I. Morfologia i cytologia.—Azotobacter
        Studien. I. Morphologie und Cytologie. *In* Bul. Internat. Acad.
        Sci. Cracovie, Cl. Sci. Math. et Nat., 1912, s. B, no. 3, p. 87–174, pl. 7–9.
(16) SACKETT, W. G.
    1911. Bacteriological studies of the fixation of nitrogen in certain Colorado
        soils. *In* Colo. Agr. Exp. Sta. Bul. 179, 42 p., 5 fig., 2 pl.

Azotobacter chroococcum

Fig. 1.—Vital-stained preparation 37 days old.

Fig. 2.—An 18-hour-old culture stained by the Guignard stain, showing strongly dyed protoplasmic granules.

Fig. 3.—A 65-hour-old culture stained by the Guignard method.

Fig. 4.—A 9-day-old culture stained by the Guignard method. The cytoplasmic matrix is here distinctly visible.

Fig. 5.—A 65-hour-old culture stained progressively by the Heidenhain method showing some empty sheaths of the peculiar zooglea masses detected by Beijerinck and Krzemieniewsky. The partition walls are visible.

Fig. 6.—An 18-hour-old culture on mannit agar, showing the life cycle of the organism. Stained by the Guignard method.

Fig. 7.—An 18-hour-old culture on mannit agar, showing the life cycle of the organism. Stained with methylene blue, 1 to 1,000.

Fig. 8 and 8a.—Cells drawn from an 18-hour-old culture on mannit agar. Stained by the Guignard method.

10 μ.
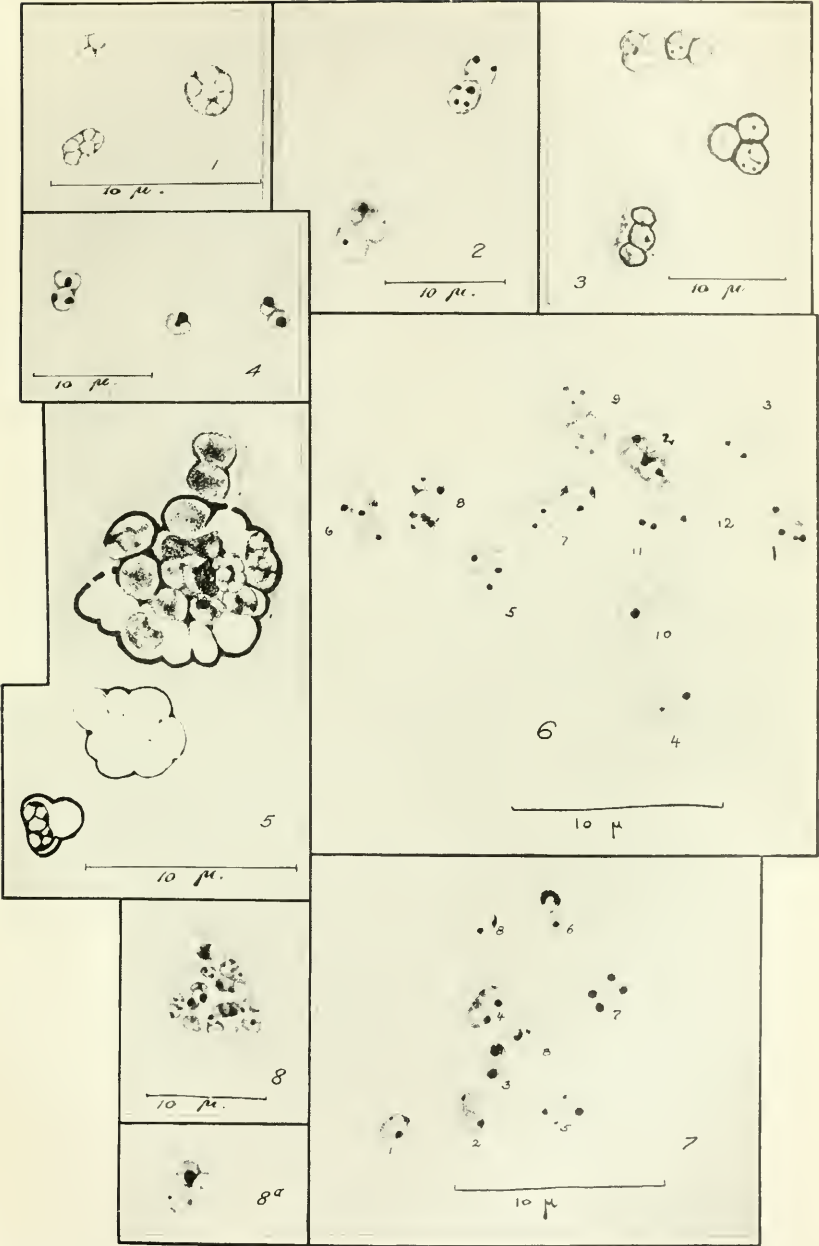
240.²

Azotobacter chroococcum: Cells drawn at random from a 65-hour-old culture on mannit agar.   Stained by the Guignard method.

91007°—15——4

PLATE XXXIII

Azotobacter chroococcum

Fig. 1.—Photomicrograph of a 65-hour-old culture stained by the Guignard method.

Fig. 2, 3.—Photomicrograph of a 65-hour-old culture stained by the Heidenhain method.

Fig. 4.—Photomicrograph of a 2-day-old culture stained with methylene blue, 1 to 1,000.

l (v
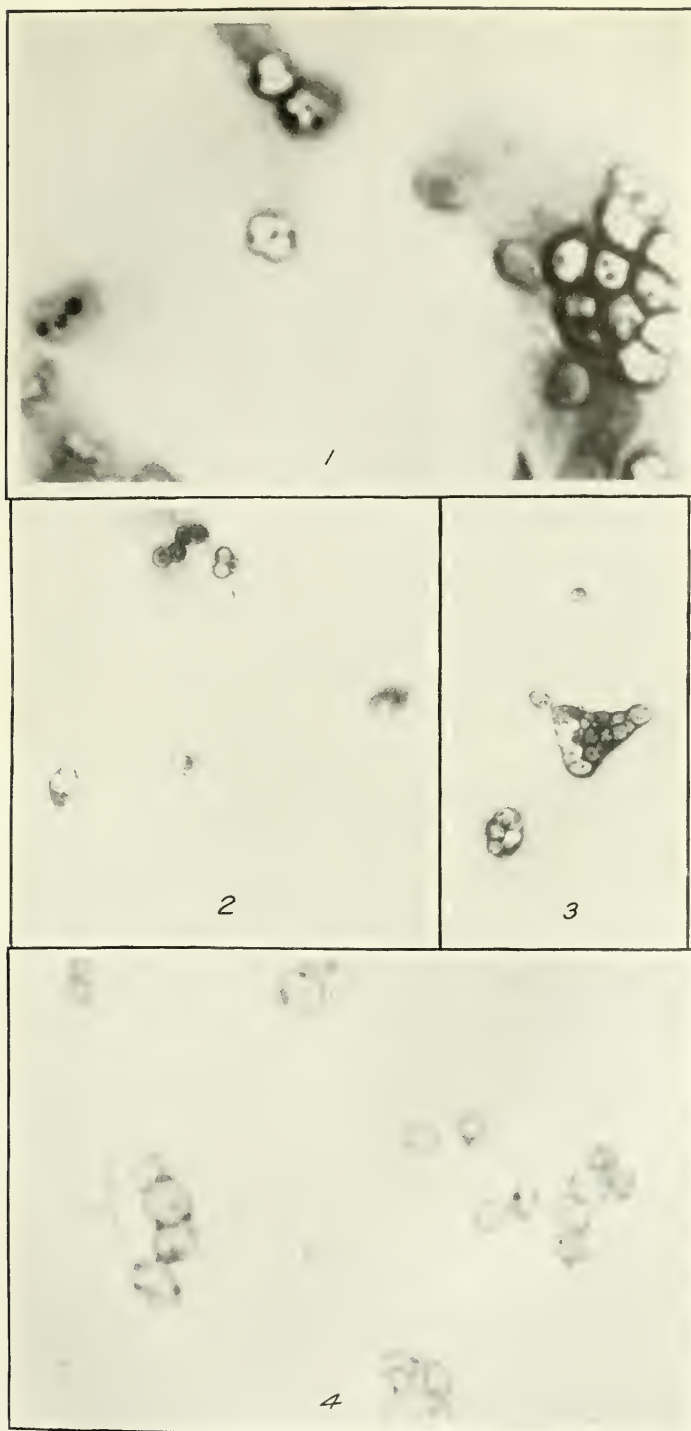
INFLUENCE OF SOIL MOISTURE UPON THE RATE OF INCREASE IN SUGAR-BEET ROOT-LOUSE COLONIES

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INTRODUCTION

In a study of the sugar-beet root louse (*Pemphigus betae* Doane) carried on by the Montana Agricultural Experiment Station as an Adams project since 1909, it was early recognized that soil moisture was an important factor in controlling the rate of increase in root-louse colonies. By means of general field observations, insectary experiments, and field tests a considerable mass of data concerning this point has been collected; and, because of their bearing on methods of control, they are now published.

GENERAL FIELD OBSERVATIONS

Outside of the sugar-beet (*Beta vulgaris*) fields the subterranean form of the root louse is most commonly found upon lamb's-quarters (*Chenopodium album* L.) growing in dry situations. The largest and most flourishing colonies are to be found where this weed has pushed its way through a ground covering of dry barnyard manure, pine needles, or other material which provides a comparatively dry medium for the ramification of the smaller rootlets. Lamb's-quarters growing in continuously damp situations makes a larger and more succulent growth, but is rarely heavily infested.

In the sugar-beet fields it has been noted many times that root lice are most abundant and that root-louse injury first appears where the soil is the driest. A striking illustration of this was noticed in a large sugar-beet field that was cut diagonally by a depression in which the ground remained moist without irrigation the entire summer. When the field was visited in October, there was a sharp line of demarcation between the moist soil of the depression and that of the general level of the beet field, which at that time was quite dry. Sugar beets were making a fine growth in the moist soil and not a root louse could be found upon them, while in the drier soil around the borders of the depression nearly every sugar beet was very heavily infested.

INSECTARY EXPERIMENTS

In June, 1911, insectary experiments were begun for the purpose of determining the influence of moisture upon the rate of increase in sugar-beet root-louse colonies.

Sugar-beet plants 3 inches in height were transplanted singly into 8-inch pots. After they had started to make a new growth, the earth was pushed away from a portion of the taproot and eight small lice in the first instar and two adults were placed upon each plant. Thirty plants were infested in this manner.

They were divided into three lots and watered as follows: Lot 1, subirrigated every other day from June 15 to August 15; lot 2, subirrigated every day from June 15 to August 15; lot 3, watered from above every day from June 15 to August 15. So far as practicable, the water applied any one day was the same in amount for all plants, but, because of the unequal moisture-retaining capacity of the soil in different pots, this was not always advisable. In general, no plants were kept wet enough to seriously retard their growth, and none were allowed to suffer for lack of moisture. All of the pots were set in large saucers, and, where the plants were subirrigated, the water was poured into the saucer and taken up by the plant as needed. On August 15 the soil in each pot was minutely examined, and all root lice that could be recovered were counted.

The data obtained are shown in Table I.

TABLE I.—*Record of sugar-beet root-louse increase under different soil-moisture conditions. First insectary experiment*

Number of plants and moisture conditions.	Initial infestation.	Total infestation at end of 2 months.
10 plants subirrigated every other day	100 lice, 10 per plant.	4,554
10 plants subirrigated every day	.....do	1,760
10 plants watered from above every day	.....do	214

Upon plants subirrigated every day 100 root lice increased to 1,760 in two months. Upon plants receiving the same amount of water from above 100 root lice increased to only 214 in the same length of time. This decided difference was probably due to the fact that in the subirrigated pots the soil was comparatively dry to a depth of several inches below the surface. It was from this drier soil that most of the root lice were recovered. Where the water was added from above, the soil was soaked throughout each day, apparently bringing about conditions very unfavorable to root-louse increase.

Upon plants subirrigated every other day 100 root lice increased to 4,554 in two months. By far the greatest number of lice was produced upon the sugar beets grown under the driest conditions.

In January, 1912, practically the same experiment was repeated, the only difference being that instead of applying a certain amount of water on certain days water was applied whenever necessary to maintain the soil conditions desired. Thirty plants, infested as before, were divided

into three lots and watered as follows: Lot 1, subirrigated so as to keep the top 2 inches of soil dry; lot 2, subirrigated so as to keep surface soil slightly moist; lot 3, watered from above so as to keep soil very moist throughout. At the end of two months all root lice that could be recovered were counted. The results are given in Table II.

TABLE II.—*Record of sugar-beet root-louse increase under different soil-moisture conditions. Second insectary experiment*

Number of plants and moisture conditions.	Initial infestation.	Total infestation at end of 2 months.
10 plants subirrigated; 2 inches of dry soil at top....	100 lice, 10 per plant.	7,027
10 plants subirrigated; surface soil kept slightly moist.	.....do.............	750
10 plants watered from above; soil kept very moist throughout.	.....do.............	211

In this experiment root lice living upon sugar beets in the driest soil again showed the highest rate of increase, 100 increasing to 7,027 in two months.

Combining the data from the two insectary experiments, the following statement may be formulated:

> 200 sugar-beet root lice in rather dry soil increased in two months to 11,581.
> 200 sugar-beet root lice in rather moist soil increased in two months to 2,510.
> 200 sugar-beet root lice in very moist soil increased in two months to 405.

FIELD EXPERIMENTS IN MONTANA

During the summer of 1914 experiments for the purpose of determining the influence of irrigation upon the reproductive power of the sugar-beet root louse were carried on at the following places in Montana: Huntley Experimental Farm, Huntley, Yellowstone Valley; Montana Experiment Station farm, Bozeman, Gallatin Valley; Billings Sugar Co.'s experimental farm, Edgar, Clark Fork Valley. Very different conditions prevailed at each place, and the experiments are therefore reported under separate heads.

IRRIGATION EXPERIMENT AT HUNTLEY

Six one-tenth-acre plots of sugar beets located on the experimental farm of the Bureau of Plant Industry at Huntley were used in the experiment. Mr. Dan Hansen, farm superintendent, was directly in charge of the experiment, and his hearty cooperation at all times is here acknowledged. The plots were about 3 miles from the nearest cottonwood trees (*Populus* spp.) and were, therefore, not in a position to become so heavily infested as were the plots at Edgar, which were bordered by a cottonwood grove.

The months of July and August were very dry, thereby making it possible to maintain the desired moisture conditions in both the wet and the dry plots. The rainfall in inches from June 15 to September 15 is given in Table III.

TABLE III.—*Record of rainfall at Huntley, Mont., from June 15 to Sept. 15, 1914*

Date.	Precipitation.	Date.	Precipitation.
	Inches.		*Inches.*
June 16.....................	0. 25	July 10.....................	0. 04
19.....................	. 22	11.....................	. 01
20.....................	. 02	Total.................	. 05
23.....................	. 02		
25.....................	1. 23	Sept. 12.....................	. 64
26.....................	. 12	Total.................	. 64
27.....................	. 33		
Total...............	2. 19	Total for season	2. 88

The sugar beets were grown according to ordinary practice, except in the matter of irrigation. Three plots were irrigated, for the purpose of keeping the soil fairly moist at all times. To accomplish this, water was applied five times: July 3, July 10, July 18, July 30, and August 24. Cultivations were given as follows: June 10, June 20, July 16, and July 25. Alternating with these plots were three which were allowed to become quite dry between irrigations; however, they suffered no more from lack of moisture than do many beets grown under ordinary farm practice. The dry plots were irrigated twice: July 22 and August 20. Cultivations were given on June 10 and July 2.

Beginning on September 17 each beet was examined for root lice and its condition recorded as it was pulled from the ground. Beets bearing from 1 to approximately 25 lice were classed as "slightly infested"; beets more than slightly infested but having no more than half of their surface covered with root lice and their waxy secretions were classed as "badly infested"; beets more than half covered were classed as "very badly infested." Badly and very badly infested sugar beets were considered as injuriously infested. A record was also made of the sugar content and the yield in pounds. The sugar analyses were obtained from samples sent to the factory according to the routine method.

The combined results from the six plots are given in Table IV.

At harvest time there was little difference in the appearance of the beet foliage on the various plots, and one not familiar with the experiment could not have distinguished between the plots which received five irrigations and those that received only two. However, an examination of the roots showed a considerable difference in the percentage of beets infested, as well as a difference in sugar content and weight. Under the drier conditions the infestation was 64.7 per cent, while where the soil

was kept moist the infestation was reduced to 31.4 per cent. Moreover, the sugar beets that received the greater number of irrigations yielded the highest in sugar and in weight.

TABLE IV.—*Record of sugar-beet root-louse increase under different soil-moisture conditions. Huntley irrigation experiment*

Number of plots and number of irrigations.	Condition of sugar beets at harvest.						Average sugar content.	Total yield.
	Number of plants uninfested.	Number of plants slightly infested.	Number of plants badly infested.	Number of plants very badly infested.	Percentage of infestation.	Percentage injuriously infested.		
							Per cent.	*Pounds.*
3 plots, 2 irrigations............	2,659	4,343	457	87	64.7	7.2	14.3	7,405
3 plots, 5 irrigations...........	5,196	2,053	245	84	31.4	4.3	16.4	8,984

IRRIGATION EXPERIMENTS AT BOZEMAN

Four one-quarter-acre plots of sugar beets located on the Montana Experiment Station farm were used for the experiment. There were numerous cottonwoods within a mile of the plots, and two years previously sugar beets in the same location had been heavily infested with lice.

The sugar beets were not harvested until October 18 and were subjected to considerable rain during September and early October. The precipitation was considerably greater than at Huntley and Edgar (Table V).

TABLE V.—*Record of rainfall at Bozeman, Mont., from June 15 to Oct. 15, 1914*

Date.	Precipitation.	Date.	Precipitation.	Date.	Precipitation.	Date.	Precipitation.
	Inches.		*Inches.*		*Inches.*		*Inches.*
June 20....	0.49	July 13.....	0.01	Sept. 5.....	0.07	Oct. 1....	0.01
21....	1.00	14.....	.05	12.....	.76	3....	.40
22....	.02	15.....	.02	13.....	.21	4....	.10
26....	.35	20.....	.17	14.....	.01	5....	.13
29....	.11	31.....	.01	15.....	.57	6....	.38
				18.....	.09	7....	.22
Total....	1.97	Total....	1.28	20....	.91	10....	.50
				21....	.68	11....	.10
July 1.....	.02	Aug. 3....	.09	27.....	.09	12....	.02
2.....	.11	23....	.02			13....	.07
5.....	.03			Total....	3.39		
10.....	.51	Total.....	.11			Total....	1.93
11.....	.35						
						Total for season ...	8.68

The beets were grown according to ordinary farm practice, except in the matter of irrigation. They were put in late, however, and a very poor stand was obtained, which accounts for the low yield. Two plots

were irrigated for the purpose of keeping the soil fairly moist at all
times. To accomplish this, water was applied three times: July 3,
August 10, and August 25. Cultivations were given as follows: June 17,
June 30, July 8, and July 20. Two alternate plots were allowed to
become quite dry and were irrigated only once during the summer:
August 10. They were cultivated on June 17 and July 22. On October 18
each beet was examined for root lice, as in the Huntley experiment.

The combined results from the four plots are given in Table VI.

TABLE VI.—*Record of sugar-beet root-louse increase under different soil-moisture con-
ditions. Bozeman irrigation experiment*

Number of plots and num-ber of irrigations.	Condition of sugar beets at harvest.						Total yield (in pounds).
	Number of plants unin-fested.	Number of plants slightly infested.	Number of plants badly infested.	Number of plants very badly infested.	Percent-age of infesta-tion.	Percent-age injuri-ously infested.	
2 plots, 1 irrigation . . .	3, 304	3, 124	0	0	48. 6	0	6, 510
2 plots, 3 irrigations. . .	8, 612	830	0	0	8. 8	0	8, 680

Not a beet in any of the plots was injuriously infested at the time of
harvest. It is hard to understand why the infestation in all plots was
so slight, unless we take into consideration the factor of rainfall. Fre-
quent rains during September and early October kept all the plots quite
wet and may have resulted in killing many of the lice.

The experiment, however, showed a marked difference in the number
of slightly infested plants on the wet and the dry plots. In the plots
irrigated only once 48.6 per cent of the plants were infested, while in
the plots irrigated three times the infestation was reduced to 8.8 per
cent.

IRRIGATION EXPERIMENT AT EDGAR

Four plots, each containing 0.45 acre, were used. They were located
on the Billings Sugar Co.'s experimental farm and were in charge of Mr.
Hans Mendelson, whose friendly cooperation made the experiment at
this place possible.

The plots were bordered by a grove of cottonwoods, in which many of
the trees bore thousands of galls of the sugar-beet root louse, and during
the summer migration period the winged lice could be seen flying in large
numbers from the trees to the beets. Upon some plants as many as 8
migrants were seen at one time. Each migrant on an average produces
10 young, which descend to the beet roots; and, when it is stated that
in insectary experiments 10 lice have been known to increase to 2,150
in two months, it can readily be understood why the infestation was so
severe.

The rainfall from June 15 to September 30 is given in Table VII.

TABLE VII.—*Record of rainfall at Edgar, Mont., from June 15 to Sept. 30, 1914*

Day.	Precip-itation.	Day.	Precip-itation.	Day.	Precip-itation.	Day.	Precip-itation.
	Inches.		*Inch.*		*Inch.*		*Inch.*
June 20....	0. 30	July 10....	0. 40	Aug. 16....	0. 20	Sept. 15....	0. 15
26....	0. 85			19....	. 11	20....	. 80
29....	. 26	Total	. 40				
				Total	. 33	Total	. 95
Total	1. 41						
						Total for season ...	3. 09

The sugar beets at Edgar were not thinned, as small beets to be used for seed growing the following year were desired. This accounts for the large number of beets reported upon, over 61,000 in this one experiment. Two plots were so irrigated that the soil was kept fairly moist for the greater part of the growing season. Water was applied June 23, July 9, August 10, and September 2. Cultivations were given as follows: May 30, June 9, July 13, and July 27. Two alternate plots were allowed to become quite dry between irrigations, but they suffered no more from lack of moisture than do many sugar beets under ordinary farm practice. They were irrigated on June 9 and August 10. Cultivations were given on May 30, June 9, and July 13.

Beginning on October 2 each beet was examined for root lice, as in the Huntley and Bozeman experiments. Sugar analyses were made at Edgar by Mr. J. F. Jarrel.

The combined results from all plats are given in Table VIII.

TABLE VIII.—*Record of sugar-beet root-louse increase under different soil-moisture conditions. Edgar irrigation experiment*

Number of plots and number of irrigations.	Condition of sugar beets at harvest.						Average sugar content (per cent.)	Total yield (in pounds).
	Number of plants uninfested.	Number of plants slightly infested.	Number of plants badly infested.	Number of plants very badly infested.	Percentage of infestation.	Percentage injuriously infested.		
2 plots, 2 irrigations..........	1, 265	19, 606	5, 493	3, 565	95. 7	30. 2	16. 1	11, 672
2 plots, 4 irrigations..........	9, 896	18, 186	2, 440	700	68. 0	10. 0	16. 5	13, 169

At the time of harvest the percentage of infestation was very high in all plots. In the plots irrigated only twice 95.7 per cent were infested, 30 per cent being injuriously infested. In the plots irrigated four times the total infestation was reduced to 68 per cent, and only 10 per cent were injuriously infested. The wet plots show a slightly better yield in sugar and a decidedly better yield in weight.

Several points of interest which do not show in Table VIII were especially noticeable at Edgar. There, as at Huntley and Bozeman, the irrigation water was not always distributed evenly over the plots, and some of the higher spots generally remained fairly dry, even in the plots that were supposed to be the wettest. When the beets were pulled, such areas were noticeably the most heavily infested. Had it been possible to soak thoroughly all of the surface soil in the wet plots at each irrigation, the infestation in such plots would have been in all probability still further reduced.

One of the wet plots was adjacent to an irrigating ditch which carried more or less water all summer. Moisture seeped from the ditch into the beet field, and at the time the beets were pulled the soil in the first and second rows from the ditch was noticeably more moist than in the remainder of the plot. It is highly significant that each of these rows contained more plants entirely free from root lice than did any of the other 78 rows included in the Edgar experiment. The infestation in the first six rows from the ditch is given in Table IX.

TABLE IX.—*Relative root-louse infestation in six rows of sugar beets close to an irrigation ditch at Edgar, Mont.*

No. of row from ditch.	Condition of sugar beets at harvest.			
	Number of plants uninfested.	Number of plants slightly infested.	Number of plants badly infested.	Number of plants very badly infested.
1	552	393	24	2
2	496	394	32	7
3	223	525	76	18
4	244	456	93	14
5	114	554	102	21
6	233	470	116	11

SUMMARY OF FIELD EXPERIMENTS

In summing up the results of the irrigation experiments at Huntley, Bozeman, and Edgar, it may be said that sugar beets grown under rather moist conditions were the least infested with root lice and yielded the highest both in sugar content and in tonnage. By combining the results from the three experiments Table X has been constructed.

TABLE X.—*Combined records of sugar-beet root-louse increase under different soil-moisture conditions. Irrigation experiments at Huntley, Bozeman, and Edgar, Mont.*

Moisture condition.	Number of beets grown.	Condition of sugar beets at harvest.						Average sugar content (per cent.)	Total yield (in pounds).
		Number of plants uninfested.	Number of plants slightly infested.	Number of plants badly infested.	Number of plants very badly infested.	Percentage of infestation.	Percentage injuriously infested.		
Fairly dry....	43,901	7,226	27,073	5,950	3,652	83.5	21.9	15.2	25,587
Fairly moist..	48,342	23,704	21,069	2,655	784	50.7	7.1	16.4	30,833

RELATION OF SOIL MOISTURE TO SUGAR-BEET ROOT-LOUSE CONTROL

From the results obtained by field observation, insectary experiments, and irrigation tests it seems safe to assume that soil moisture is a very important factor in controlling the rate of increase in colonies of the sugar-beet root louse. While root lice were in no instance entirely controlled by irrigation during the first year's experiments, their number was greatly reduced, and it is hoped that a system of irrigation may be worked out that will reduce root-louse injury to a negligible amount. It is expected that such a system will not interfere with the approved sugar-beet cultural methods, but that in the light of experiments reported in this paper it will rather tend to increase both the sugar content and the tonnage, and will thus perhaps more than pay for the extra labor it may demand. The principal immediate source of root-louse infestation of sugar beets is the cottonwood (*Populus balsamifera* L. and *P. angustifolia* James), upon which the root louse develops galls in the spring. During the latter part of June and early July some of the numerous migrants that have developed within the galls fly to sugar beets, where they deposit living young, which descend to the roots and start new colonies. In studying the life history of the sugar-beet root louse in the insectary it has been found very difficult to induce the progeny of the migrants to colonize upon sugar beets growing in soil the surface of which is at all moist. The only successful attempts in colonization have been where sugar beets were subirrigated and several inches of dry soil were kept at the surface. It therefore seems highly important that sugar-beet fields should not be allowed to dry out during the period when the sugar-beet root louse is migrating from the cottonwoods to the beet fields. All too frequently this is just the time when in ordinary farm practice the beet fields are allowed to become quite dry. The June rains cease, and by July 1 in an average year nearly all the beet fields in the Yellowstone Valley need water. Many times water is purposely withheld at this time

because of the mistaken idea that if the fields are allowed to dry out the sugar-beet plant will send its taproot deeper into the soil in search of moisture and thus produce a better-shaped beet. Extremely dry conditions at this time not only check the growth of the plant but offer ideal conditions for the starting of root-louse colonies. The ground is generally cracked open about the base of the plant and the young lice deposited upon the leaves by the migrants have no difficulty in establishing themselves upon the fiber rootlets in the comparatively dry soil, where all conditions are favorable for rapid growth and multiplication. Fortunately the best authorities on sugar-beet culture are urging against allowing the beet fields to become dry at this time, not primarily because of the possibility of root-louse infestation, but because of the general welfare of the crop. Mr. Hans Mendelson, scientist for the Billings Sugar Co., has stated to the writer several times that all of that company's irrigation experiments have shown that early and frequent irrigations produce the highest sugar content and tonnage. In a recent letter he states, "All our experiments in early irrigation have shown that irrespective of aphis it is the right treatment." Thus, by irrigating early, before the fields become dry, the chances for root-louse infestation are reduced and the best conditions for plant growth are secured.

It is also important that plenty of soil moisture be maintained throughout the growing season. Some of the myriads of young lice produced by the migrants from the cottonwoods are almost sure to become established even in fields that are well irrigated. The presence of sufficient soil moisture will tend to retard their increase and by promoting a vigorous plant growth will enable the sugar beets to withstand better the root-louse attacks. A scarcity of soil moisture results in a rapid multiplication of the few root lice that may be in the soil, and plants suffering for lack of moisture are in very poor condition to withstand the drain of root-louse attacks.

The details of this system, such as the number of irrigations per season, the quantity of water to be applied at one irrigation, and the question of cultivation, have not been thoroughly worked out, and the problem will demand several seasons' work before definite recommendations can be made. However, the principle is believed to be sound, and at present irrigation apparently offers the most effective and the most practical method of controlling the sugar-beet root louse.

A NEW LEAF AND TWIG DISEASE OF
PICEA ENGELMANNI

[A PRELIMINARY REPORT]

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The doubt expressed by some mycologists that *Herpotrichia nigra* Hartig and *Neopeckia coulteri* (Pk.) Sacc. are distinct species should be entirely dispelled by the recent article by Sturgis.[1]

These species are chiefly distinguished by the color and form of their spores. The spores of *N. coulteri* when mature are blunt-elliptical, dark-brown, 1-septate, conspicuously constricted at the septa, measuring from material at hand 19.8 to 28.9 by 9.5 to 10μ (Pl. XXXIV, fig. 1, A). Those of *H. nigra* when mature are elliptical, "pale to darker olivaceous-brown," 3-septate. They are not so dark in color, nor are they so conspicuously constricted at the septa as those of the former species. These spores measure from material at hand 21.8 to 31.8 by 7.2 to 9.0μ (Pl. XXXIV, fig. 1, B).

The microscopical characters of the spores of these species are seen to be quite distinct. Throughout the examination of a large amount of material from all parts of the Northwest and from many hosts these characters were found to be constant. It is practically impossible to distinguish the two species by their gross appearance in nature. The mycelium of both involves the leaves and stems of their hosts in a felt-like mass, resulting in the death of the parts infected. Occasionally the mycelium of *N. coulteri* has a lighter color than that of *H. nigra* when in mass, but either species may vary in this respect with age and long exposure. As a rule, the species may be readily recognized by their choice of hosts. When not in contact or intermingled with others, the host plants selected by *H. nigra* invariably belong to some species of the genera Abies, Juniperus, Picea, Libocedrus, or Tsuga.[2] The writer has collected specimens on the above genera and also on Thuya and Taxus. Hartig[3] reports *H. nigra* on *Pinus montana* in Europe. With the exception of very special instances *N. coulteri* is always found associated with the genus Pinus. Occasionally both

[1] Sturgis, W. C. Herpotrichia and Neopeckia on conifers. *In* Phytopathology, v. 3, no. 3, p. 152–158, pl. 12–13. 1913.

[2] Hedgcock, G. G. Notes on some diseases of trees in our national forests. *In* Phytopathology, v. 4, no. 3, p. 181–183. 1914.

[3] Hartig, Robert. Trichosphaeria parasitica und Herpotrichia nigra. *In* Hedwigia, Bd. 27, Heft 1, p. 12–15. 1888.

these fungi spread to the leaves of an adjacent tree which normally is never selected as a host. This occurs when the branches and leaves of the regular host are intermingled or in contact with those of the other. The mycelium in such cases may be simply crowded over and may continue to draw its nourishment from its regular host. Instances of this kind have been noted where *H. nigra* spread from *Abies lasiocarpa* to *Phyllodoce empetriformis* (Smith) D. Don. Whether the mycelium actually penetrates the tissues of the leaves of the borrowed host has not been determined. The epiphytism of the fungus, however, is sufficient to cause the death of the leaves covered by its mycelium.

So selective are these two interesting fungi with regard to their hosts, it has always been a field practice of the writer to refer them to their respective genera by this fact alone. On the examination of several collections of what seemed to be *H. nigra* on *Picea engelmanni* from Marble Mountain, St. Joe National Forest, Idaho, it was found that the fungus was not this species but another of the same genus. This led to a further examination of the same material and also of other collections from various regions of the West which were sent in from the field labeled "*Herpotrichia nigra*." The fungus on a large part of these collections on species of Picea was found to be quite different in its microscopic characters from *H. nigra*. Although the gross appearance of the mycelial mat was the same (Pl. XXXIV, fig. 2), the mature spores were uniformly 5-septate, and were scarcely constricted at the septa, which were prominent and fairly thick (Pl. XXXIV, fig. 1, *C*). A large amount of material was examined, and the 5-septate spore was found to be as characteristic a feature of this fungus as is the 1-septate spore for *N. coulteri* and the 3-septate spore for *H. nigra*. Mature apothecia of all three fungi were crushed together and mounted on the same slide. Plate XXXIV, figure 1, *A*, *B*, and *C*, are reproduced from camera-lucida drawings of this material and show the proportionate size and character of the asci and spores for all three species.

Since the fungus originally collected on *Picea engelmanni* from Marble Mountain, Idaho, does not agree with *H. nigra* Hartig or with any other known species, it is described as new:

Herpotrichia quinqueseptata, n. sp.

Perithecia gregarious or scattered, spherical, 0.19 to 0.43 mm. in diameter, partially embedded in a dark-brown subiculum 0.15 to 0.48 mm. thick, more often free, ostiola not prominent. Asci cylindrical or slightly fusiform, 99.8 to 137.6 by 14.1 to 16.5μ. Paraphyses filiform, fugacious. Ascospores irregularly biseriate in the ascus, fusoid or long elliptical, sometimes slightly curved; when mature, 5-septate, may be slightly constricted at the septa, light brown, 28.3 to 33.8 by 7.6 to 9.05μ.

Type locality.—Marble Mountain, St. Joe National Forest, Idaho.

Habitat.—Living twigs and leaves of *Picea engelmanni*.

Type material has been deposited in the Office of Investigations in Forest Pathology and in the Pathological Collections, Bureau of Plant Industry, Washington, D. C.

All three of the foregoing species are truly alpine in habit and are not usually found growing normally below an elevation of 5,000 or 6,000 feet. This habit, of course, varies with the latitude. Moreover, they are very common, and from the writer's experience they cause considerable damage to alpine forests, particularly to the younger trees. Young seedlings 4 to 8 years old have been found infected, and old trees frequently succumb to their ravages. In some regions of the Northwest on northern exposures, entire stands when composed mainly of even-aged alpine fir are frequently so generally infected by *H. nigra* as to appear ragged and bare. A sample acre taken on a north slope of Mt. Casey (Selkirks, northern Idaho) at an elevation of 6,735 feet showed 95 per cent of the alpine firs to be infected by this fungus.

The influence of these fungi on their hosts is likewise discernible in the gradual falling off of the annual increment. This causes a sharp contrast in the radial dimensions of the annual rings, even in the finely layered condition of the wood of alpine trees. The dense mat of brown or black mycelium (Pl. XXXIV, fig. 2), often of sufficient thickness and extent to completely spread over entire twigs, burying the leaves entirely in its mass, is enabled to bring about certain phenomena of a very unusual nature. It has been found by actual experiment that this mycelial mat influences the temperature of the enveloped leaves in the same manner as any dark covering acts on the bulb of an air thermometer. The fungus acting as a pronounced epiphyte may thus be enabled through a slight rise in temperature to incubate its own mycelia within the tissues of the leaf and hence may hasten its parasitic activities. Probably the spread of the epiphytic mycelium to leaves not infected internally at the time, as sometimes occurs, is accompanied by reactions of a physiological nature that are highly injurious and pave the way for the advance of the mycelium into the tissues of the leaf. The spread of the mycelium over young growth, from the time the snow disappears in the spring to early fall, is fairly rapid. Branches of alpine fir 2 feet in length that were infected at their tips in the early spring have had their entire leaf surface destroyed by October of the following year.
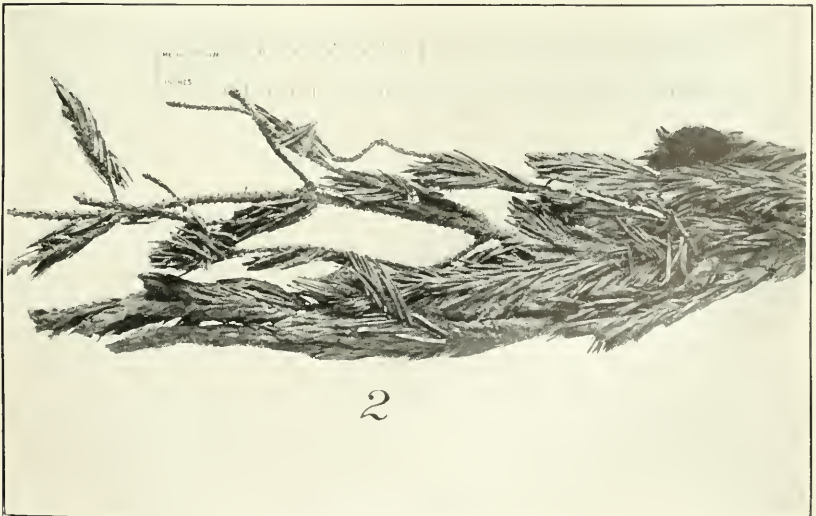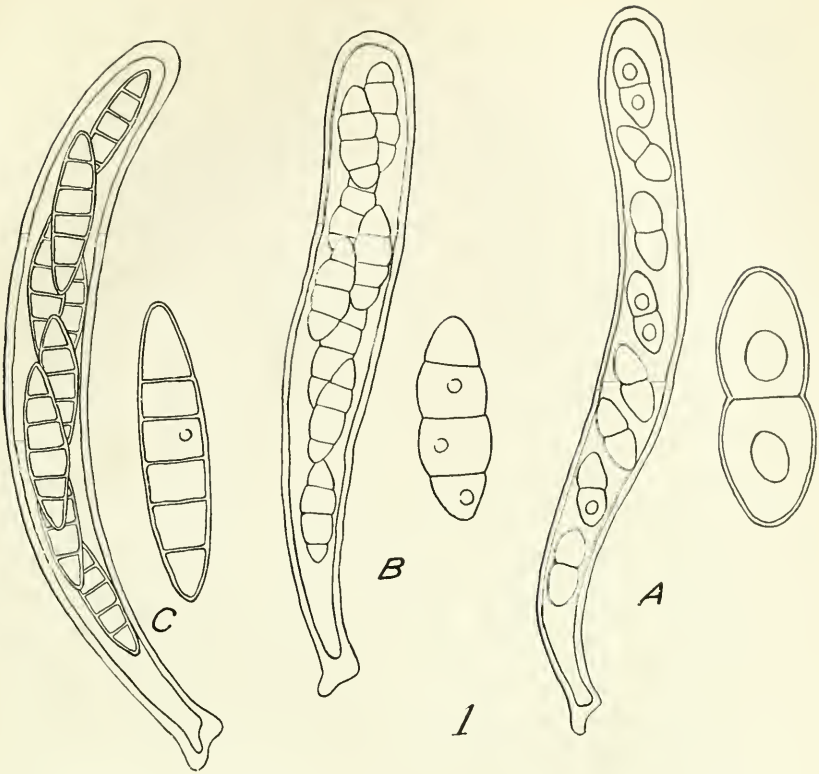
SOME SUGAR-CANE ROOT-BORING WEEVILS OF THE WEST INDIES

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INTRODUCTION

Sugar-cane (*Saccharum officinarum*) growers throughout the world find among their most serious enemies the weevils which bore in the subterranean portions of the stalks and the root crown. The methods of cultivation and propagation in vogue in the sugar-cane industry make an attack of this nature more serious than injury to another part of the plant.

In the West Indies one of the most important groups of economic weevils is the genus Diaprepes, which is mainly confined to the periphery of the Caribbean Sea. The present paper deals with the weevils of this genus which attack sugar cane and which are popularly known as sugar-cane root borers. The writer's purpose is to straighten out the difficult nomenclature, to point out the dangerous nature of the injury by the species treated, and so to describe the various forms that quarantine agents may readily detect them.

These weevils are so variable in color, shape, and markings that it is extremely difficult to come to a definite decision as to their specific limitations. Many names have been used which can only be considered of varietal significance or as mere synonyms. The same species is likely to be known by different names in different islands. It is therefore necessary to go a little deeply into the description of the various forms and to trace the blending from one form to another through the islands. For this reason the technical matter must receive more attention than the biological and economic data belonging with it.

GENUS DIAPREPES

THE ADULT

The genus Diaprepes Schönherr contains many species found in the West Indies, Mexico, and Central and South America. It is typically tanymecine in the possession of distinct ocular vibrissæ on the prothorax in the majority of species, although occasional specimens of undoubted Diaprepes are found without trace of the vibrissæ. The genus is distinguished by the peculiar irregularities of the elytral striæ, which num-

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ber over ten. The species attacking sugar cane belong to the group having three rostral carinæ and are to be separated as follows:

Classification of sugar-cane species and varieties of Diaprepes

I. Beak with three rostral carinæ but not otherwise rugose; first funicular joint very much smaller than second; densely squamose, more or less denuded in vittæ. Prothorax not conspicuously transverse, usually a little longer than wide; vibrissæ distinct; body with a lateral yellow or pink vitta.........*spengleri* Linnaeus.
  1a. Median rostral carina with fovea near apex; elytra without denuded intervals; undersides evenly pubescent; pubescence consisting principally of flat scales....................................variety *marginatus* Olivier.
  1b. Median rostral carina without fovea but with transverse carina at same point connecting with the lateral carinæ; elytra with denuded intervals; pubescence throughout consisting of flat and upright scales.
    2a. Elytra with only a single row of punctures between the two humeral denuded intervals.
      3a. Fifth interval denuded at base............variety *comma* Boheman.
      3b. Fifth interval denuded at base, and third on disk.
                               variety *spengleri* Linnaeus.
      3c. Third and fifth intervals denuded at base. variety *abbreviatus* Olivier.
      3d. Third to tenth intervals denuded....variety *denudatus*, new variety.
    2b. Elytra with two rows of punctures between the two humeral denuded intervals; third and fifth intervals denuded at base.
                              variety *festivus* Fabricius.
II. Beak with three rostral carinæ and closely recticulately rugose; first funicular joint very nearly as long as second; sparsely squamose.......*famelicus* Olivier.

DIAPREPES SPENGLERI LINNAEUS

No species hitherto studied by the writer has shown such a wide range of variations in colors and markings as *Diaprepes spengleri* Linnaeus. The two sexes differ so much in shape that the male of one variety and the female of another would easily pass as very distinct species. The structural and sculptural differences, however, are exceedingly minute, and the large series of specimens from Porto Rico, Barbados, and the intermediate islands shows that they must all be one species.

The following characters are common to all forms of the species and are therefore of specific weight in comparing this species with others in the genus:

Beak tricarinate, with indications of a transverse carina near apex; nasal plate emarginate, triangular. Antennal scrobes arcuate, passing immediately beneath the eye; scape elongate, clavate; funicle with second joint longer than first, third joint a little longer than the following, which are slightly longer than wide and subequal; club elongate, pointed, and very finely pubescent. Prothorax very irregularly confluently punctured, sparsely pubescent above, laterally densely vittate; truncate at apex, with distinct ocular vibrissæ. Scutellum subquadrate, rounded behind. Elytral striæ confused from sixth to sides. Elytra of female acute and somewhat sinuate on margin behind; elytra of male less acute, with margin convex. Last ventral segment of female triangular, of male broadly rounded behind. Tibiæ with a few small denticles.

THE IMMATURE STAGES

A sketch of an egg is given in Plate XXXVIII, figure 1, *F*.

The larvæ of weevils are most readily distinguished one from another by the folds of the body, the shape and arrangement of the spiracles, the head, and the caudal segment. Sketches of these parts are therefore given to assist in the identification of larvæ of *Diaprepes spengleri*. These drawings have been made with great accuracy with the intention of bringing out the systematic characters (Pl. XXXVII, fig. 2, and Pl. XXXVIII, fig. 1, A–E).

In the pupæ of weevils we find even better characters for separating species. The general form of the pupa and the arrangement of the ventral parts is of considerable importance (Pl. XXXVIII, fig. 2). The mouth parts of this species are very interesting and have been illustrated in Plate XXXVIII, figure 2, *B*. The arrangement of the tubercles on the dorsal segments is important, especially in the scutellar region (Pl. XXXVIII, fig. 2, *C*), and on the apical segments (Pl. XXXVIII, fig. 2, *E*). But more important than any other characters are the structures of the last three or four ventral segments (Pl. XXXVIII, fig. 2, *D*). It is hardly necessary to add much of a descriptive nature to the figures given. The spiracles of the abdomen are dark and very plain on the first to fifth segments, and very inconspicuous on the sixth to eighth segments. The thoracic spiracle is elongate and located between the prothorax and mesothorax.

VARIETIES OF DIAPREPES SPENGLERI

Merely for the convenience of designation and to retain old, well-known names the species *Diaprepes spengleri* has been arbitrarily arranged into varieties by the writer.

The variety which is presumably nearest the parent variety, *Diaprepes spengleri marginatus* Olivier, is only at hand from St. Croix. The next step in the progression of the species has been called *D. spengleri comma* Boheman, and ranges from the Dominican Republic and Porto Rico to Dominica; in other words occurring in both directions from the home of *D. spengleri marginatus*, with nearest approach to this form in a specimen from Dominica. The next form is merely an intermediate and is found in the collection only from Porto Rico. This form most nearly answers the description of typical *D. spengleri* Linnaeus. The fourth variety, *D. spengleri abbreviatus* Olivier, is at hand from Porto Rico, Montserrat, Dominica, and Barbados. There seem to be two trends of modification from this. The fifth variety, *D. spengleri denudatus*, n. var., is an extreme from a trend found in the Porto Rican material, and is at hand only from Guadeloupe. The sixth variety, *D. spengleri festivus* Fabricius, is from the branch of the fourth found in Dominica and Barbados, and is at hand from Barbados and St. Vincent.

Owing to the remarkable differences displayed in the species, a more or less detailed study follows to show why the writer has considered

these apparently unrelated forms all as one species. Over 40 different variations are at hand. The color names used are according to Ridgway's Color Standards.[1]

Material has been examined belonging to the United States National Museum, the Porto Rican Sugar Growers' Association, the Porto Rico Experiment Station, and the Imperial Agricultural Department of Barbados. Over 250 specimens of this species are now at hand, and, in order to show the trend of variation, the different forms are briefly described.

Diaprepes spengleri marginatus Olivier.

1. The impressions on the thorax are clad with flat white scales, which also form the general color of the vestiture of the body, except for a pale ocherous lateral vitta on the prothorax and elytra, and a trace of ocherous near the scutellum on the elytra. The elytra are uniformly squamose, with flat scales, without any denuded areas whatever. The material at hand consists of four specimens from St. Croix, two collected by Mr. Longfield Smith on cotton in May, 1912, and two collected by Mr. V. Hanchell on July 31, 1908. Size, 14 to 18 mm. This form is not absolutely linked to the following. Except for the lack of denudations it could not be readily distinguished from *D. spengleri festivus* (Pl. XXXV, fig. 1).

D. spengleri marginatus has been recorded by Fleutiaux and Sallé on *Chrysobalanus icaco* in Guadeloupe and was collected by Longfield Smith on cotton.

Diaprepes spengleri comma Boheman.

2a. The impressions on the thorax are clad with iridescent light blue-green and whitish scales, which also form the general color of the elytra and undersides, except for a broad lateral vitta of empire yellow on the elytra, and a touch of the same on each side of the scutellum. The basal third of the fifth interval, the basal half of the ninth interval, and the basal fourth of the tenth interval are denuded and shining black. One specimen is at hand from Romana, Dominica, collected by Mr. W. V. Tower on April 16, 1913. Size, 14 mm.

2b. The tendency in this variety is to increase the amount of the denuded area. The majority of the specimens are less greenish and have the basal half of the fifth interval, from one-half to three-fourths of the ninth, and from one-fourth to one-half of the tenth denuded. The lateral yellow vitta is distinct, but the yellow sutural spots are sometimes lacking. The material consists of one specimen from Dominica, collected by Mr. H. W. Foote in June or July (Yale Expedition, 1913); fourteen specimens from the Dominican Republic, collected by August Busck in August; five specimens from Guanica, and one from Santa Isabel, Porto Rico, collected by Messrs. E. G. Smyth and D. L. Van

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[1] Ridgway, Robert. Color Standards and Color Nomenclature. 43 p., 53 col. pl. Washington, D. C., 1912.

Dine on May 28, 1913, and October 20, 1910. The size varies from 11 to 15 mm. (Pl. XXXV, fig. 2).

2c. Another direction for the variation lies in the color of the scales. Three specimens from Guanica and Santa Isabel, Porto Rico, have the empire-yellow scutellar area and lateral vitta very distinct, but the remainder of the elytra is clad with tawny-ochraceous. These were all collected in May. Size, 11 mm.

2d. The yellow sutural spots and lateral vittæ are here replaced by shrimp pink in four specimens, which are almost white in general vesti-ture, and in two specimens, which are colored tawny-ochraceous, all from Guanica, Porto Rico. One of the latter has the deciduous pieces of the mandibles still intact. Size, 11 to 16 mm.

2e. This form completely lacks the colored lateral vitta. A specimen from Humacao, Porto Rico, collected on November 12, 1910, by Mr. D. L. Van Dine, and one from Bayamon, Porto Rico, collected by Mr. A. Busck in January, 1899, have the greenish scales predominant, while specimens from Guanica and Santa Isabel, Porto Rico, have the whitish scales pre-dominant. Size, 9 to 18 mm.

2f. The next change consists in the intensification of the green scales to shining pale yellow-green in specimens from Humacao, Barceloneta, and Canovanos.

2g. Then follows an admixture of a few ochraceous-tawny scales, especi-ally posteriorly, in a specimen collected on sugar cane at Yabucoa, Porto Rico, by Mr. D. L. Van Dine on April 20, 1911.

2h. The next modification is the strong admixture of ochraceous-tawny scales on all parts of the elytra except near the suture, while on the thorax and beneath, all the scales remain green, as found in a specimen from Bayamon, Porto Rico, collected by Mr. Van Dine on October 9, 1910. Size, 11 to 14 mm.

2i. In the final gradations of this variety the general scale coloring of the elytra is light buff to ochraceous-tawny, numerous different tones being found in the present series. In several specimens the body color is a dark Hessian brown. Two specimens from Yauco, Porto Rico, collected in May, 1912, and two collected on sugar cane by Mr. J. R. Johnston on June 11, 1911; four specimens from Santa Isabel, collected on sugar cane by Mr. Van Dine on May 30, 1911; a single specimen from Utuado, Porto Rico, in January, 1899, Mr. Busck, collector; two specimens from Barceloneta, P. R., on May 16, 1911, collected by Mr. J. R. Johnston. Size, 12 to 16 mm.

A newly reared specimen from Rio Piedras, collected on February 8, 1912, on sugar cane by Mr. J. R. Johnston, has the mandibles complete. The deciduous pieces are long, shining, curved, overlapping at tips, with edges sharp and the tips acute, with the upper surface strongly convex, and the lower surface concave. These pieces are longer than the first two funicular joints together.

D. spengleri comma occurs in the Dominican Republic, Porto Rico, and Dominica. In Porto Rico it intergrades by almost imperceptible changes into the forms here named *D. spengleri spengleri* and *D. spengleri abbreviatus*. On this island it has been taken at all times of the year at Buena Vista, Guanica, Yabucoa, Luquillo, and Santa Rita, by Messrs. D. L. Van Dine, T. H. Jones, E. G. Smyth, J. R. Johnston, and C. T. Murphy, on sugar cane, grass, jobo (*Spondias lutea*), bledo (*Amaranthus* sp.), and *Parthenium* spp., and has been found in all stages at the roots of sugar cane.

Diaprepes spengleri spengleri Linnaeus.

3. The third variety also lacks a different-colored lateral vitta, but has a short median denuded area on the third interval. This line varies from a dot to several millimeters in length, but is usually distant from the base. I take this variety to be typical *D. spengleri spengleri*. Size, 8 to 16 mm. (See Pl. XXXV, fig. 3.)

3a. The first form, with white scales only, is represented by a specimen from Yabucoa, taken on sugar cane on April 20, 1911, by Mr. D. L. Van Dine.

3b. Three specimens from the same place have the scales light yellow-green. Five in various tones of green come from Yauco and Salinas, Porto Rico, collected during May.

3c. A specimen taken on sugar cane at Fajardo on January 25, 1911, by Mr. D. L. Van Dine is light yellow-green with an admixture of tawny-ochraceous scales.

3d. By far the majority of this variety are of light buff to ochraceous-tawny in many different tones. These specimens come from Santa Isabel, Guanica, Cidea, Bayamon, Ponce, Rio Piedras, San Juan, and Maunabo, Porto Rico. One specimen from Cidea was taken by Mr. F. D. Gardner as it was injuring the orange.

3e. Two specimens from Rio Piedras of the ochraceous-tawny color were apparently collected in copulation on January 11, 1912. The female has the last ventral segment almost in the form of an isosceles triangle, with the apex narrowly rounded. The male anal segment is transverse subtriangular, with the apex broadly rounded and the surface very rugose. Two other pairs agree in these characters.

3f. This form is merely an intermediate between *D. spengleri spengleri* and the next variety. It ranges in color exactly as the preceding, but has elytral intervals 1 to 5 or 6 more or less broadly denuded. The material is from Aguadilla, Utuado, Bayamon, Fajardo, and Mayaguez in the Busck collection, and from Humacao, Guanica, Yabucoa, Casovanas, Rio Piedras, and Ponce in Mr. Van Dine's collection, most of which was taken on sugar cane. One specimen, collected at Cidea by F. D. Gardner, was injuring the orange. Size, 9 to 16 mm.

D. spengleri spengleri Linnaeus occurs only in Porto Rico. This form intergrades perfectly into both *D. spengleri comma* and *D. spengleri abbre-*

viatus in such a way as to leave no doubt that the three are one species, although the extremes appear so different. It has been taken throughout the year at Buena Vista, Guanica, Santurce, Rio Piedras, Fajardo, Yabucoa, Luquillo, Arecibo, and Cidea, by Messrs. Van Dine, Tower, Jones, Smyth, Johnston, Murphy, and Gardner on sugar cane, grass, *Mimosa* spp., *Ceratonia* spp., guava (*Psidium guajava*), avocado (*Persea gratissima*), mango (*Mangifera indica*), rose, *Spondias lutea*, *Amaranthus* spp., *Parthenium* spp., and orange (*Citrus aurantiaca*). It has been reared from the roots of the orange and sugar cane.

Eggs were obtained and described in September, 1912, by Mr. Jones. They are oblong, oval, smooth, glistening, milky white, with a rather tough membrane, and measure about 1.2 by 0.4 mm. when newly laid. In confinement the females laid the eggs between the surfaces of two leaves, the leaves being brought together and their surfaces about the egg held by an adhesive substance placed between the eggs and around the cluster. The eggs are placed in no regular pattern and are so closely pressed together that their shape is altered. When first laid, the eggs are of a uniform milky white, but within a day after being deposited clear spaces appear at both ends, being more pronounced at one end. Before hatching, these clear spaces disappear, the egg takes on a faint brownish tinge, and the mouth parts of the larva can be seen through the membrane.

The newly emerged larvæ are white, with a slight brownish tinge, have light-brown heads, and are a trifle more than 1 mm. in length. They immediately enter the ground and begin feeding on the root system of the sugar cane or other host.

Diaprepes spengleri abbreviatus Olivier.

4a. The fourth variety varies in scale color from white through green to ochraceous and sometimes has a yellow spot at the sides of the scutellum, as in the preceding varieties. It also has the lateral vitta of empire-yellow, except in the whitest specimens. The denudations of the third and fifth elytral intervals are almost equal and the intervening intervals are very narrow. The undersides are very sparsely squamose. Mr. Van Dine's material is from Anasco, Guanica, Ponce, Arecibo, and Barceloneta. Five specimens are at hand from La Yoslina, Porto Rico, collected on July 30, 1900, on shade trees. One is labeled "*Diaprepes abbreviatus*." Some specimens show a small denuded post-median line on the seventh interval. Size, 10 to 13 mm. (Pl. XXXVI, fig. 1.)

4b. This form is light-buff colored in its vesture and lacks the lateral yellow vitta. The third and fifth intervals are more widely separated. One specimen with black integument from Guanica, Porto Rico, and six specimens with Hessian-brown integument from the Island of Dominica were collected by A. H. Verrill. Size, 9 to 15 mm.

4c. The next variation has the denuded intervals more or less widely separated. It is denuded as in the preceding, but also has the ninth interval denuded almost to the apex. The vesture is in various tones of bluish green with an ocherous area on each side of the scutellum and an ochraceous-tawny lateral vitta. Three specimens are from the island of Montserrat, collected on March 1 and April 2 and 9 by Mr. H. G. Hubbard. One specimen is labeled "*Diaprepes abbreviatus.*" There are also four whitish specimens from Arecibo and Barceloneta, Porto Rico. Size, 14 to 16 mm.

4d. The next form is similarly denuded except that the denuded space on the ninth interval is united to that on the eleventh at about the middle of the elytra. The vesture is intermixed white and golden green. Two specimens from Barbados were collected by Mr. J. Morris, on May 22, 1900, and fourteen were collected by Mr. H. A. Ballou. These are all males of the form *Diaprepes spengleri festivus.*

D. spengleri abbreviatus occurs in Porto Rico, Dominica, Montserrat, and Barbados, and occurs on sugar cane in both Porto Rico and Barbados. The Porto Rican material assigned to this form does not closely resemble that from Barbados, but the variation in the Porto Rican material from the *D. spengleri* type is so great that at the other extreme specimens identical with those from Dominica and Montserrat can be found. In the same way the Montserrat material varies to the typical Barbados material, which incidentally is all male. In Guadeloupe it has been found on avocado, coffee, and *Cajanus indicus.*

Diaprepes spengleri festivus Fabricius.

5a. The fifth variety differs from the preceding by having a double row of punctures between the two humeral denuded intervals. It has an ocherous lateral vitta and spots on each side of the scutellum. The discal denudation of the seventh interval is highly variable, sometimes being connected in front to the ninth interval at about the middle of the elytra. The vesture of some specimens is white and of the others greenish. Twenty-one specimens, probably *D. spengleri festivus* Fabricius, from Barbados, mostly females, were collected by Mr. H. A. Ballou (Pl. XXXVII, fig. 1).

5b. The next form is like the preceding, but without any denudations of the ninth interval and without ochraceous markings. One specimen from St. Vincent was collected by Mr. H. H. Smith and labeled *D. spengleri* by Mr. Champion.

5c. The next variation is like the preceding, but has the denuded portion of the third interval short, discal, and the vesture uniformly buff-yellow. Four specimens from St. Vincent were collected by Mr. H. H. Smith, one of them on the castor plant (*Ricinus communis*). They are labeled *D. spengleri* by Mr. Smith.

D. spengleri festivus differs only by the humeral strial punctuation from the preceding. This form is found in Barbados and St. Vincent. It

breeds in the roots of sugar cane, Indian corn (*Zea mays*), Guinea corn (*Andropogon sorghum*), sweet potatoes (*Ipomoea batatas*), Bahama or Bermuda grass (*Capriola dactylon*), and limes (*Citrus medica acida*) in Barbados, and has been collected also on pigeon-pea (*Cajan indicum*) and Bonavist bean (*Dolichos lablab*) in Barbados and on castor plant in St. Vincent. Mr. H. A. Ballou has written considerably on this form under the name "*Diaprepes abbreviatus.*" He found the eggs in small clusters on the leaves of a variety of plants. On sugar cane the eggs are usually laid near the tips where the leaves have been split by the wind, the two portions of the leaf being stuck together over the eggs. As many as 89 eggs have been found in a cluster. The young larvæ in attacking sugar cane are first found on the fibrous roots, but as they grow larger they tunnel into the underground stem portions of the plant. Mr. Ballou places the life cycle at about a year, of which about 10 days are spent in the egg stage, 300 in the larval stage, 15 in the pupal stage, and about 20 in the adult.

Diaprepes spengleri denudatus, n. var.

6. The opposite extreme from *D. spengleri marginatus* is found in one specimen collected by Mr. H. W. Foote in Guadeloupe in June, 1913. This specimen has the greenish and whitish scales in the dorsal punctures with an ochraceous spot on each side of the scutellum, an ochraceous lateral line on the elytra, and a very white lateral vitta on the prothorax, but the second elytral intervals are the only dorsal intervals clothed with scales. Interval 1 and intervals 3 to 10 are denuded. Size, 12 mm. (Pl. XXXVI, fig. 3.)

DIAPREPES FAMELICUS

The species *Diaprepes famelicus* Olivier differs from *Diaprepes spengleri* in that the three carinæ of the beak are indistinct and the surface is very rugulose. The ocular vibrissæ are almost completely lacking. The species is black and very sparsely inconspicuously squamose. The strial punctures are larger and the striæ closer together than in *D. spengleri* (Pl. XXXVI, fig. 2).

The species is also known as *D. esuriens* Gyllenhal in some of the islands.

Material is at hand from Montserrat, Dominica, and St. Kitts.

D. famelicus attacks sugar cane in St. Kitts. Mr. H. G. Hubbard collected several specimens that were notching the leaves of the lime in Montserrat on March 31, 1894.

CONTROL OF THE SUGAR-CANE ROOT BORER

As means of control, Mr. W. V. Tower has suggested spraying with arsenate of lead the trees attacked by adults. Mr. H. A. Ballou suggests rotation of affected crops with unaffected crops, breaking up infested stumps to expose the grubs to the attack of ants and birds, and the subsequent burning of these stumps. He also recommends the hand picking of adults from April to June.

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Fig. 1.—*Diaprepes spengleri*, variety *abbreviatus*, female from Porto Rico.
Fig. 2.—*Diaprepes famelicus*, male from St. Kitts.
Fig. 3.—*Diaprepes spengleri denudatus*, new variety, male from Guadeloupe.
Drawn by Mr. Harry Bradford.
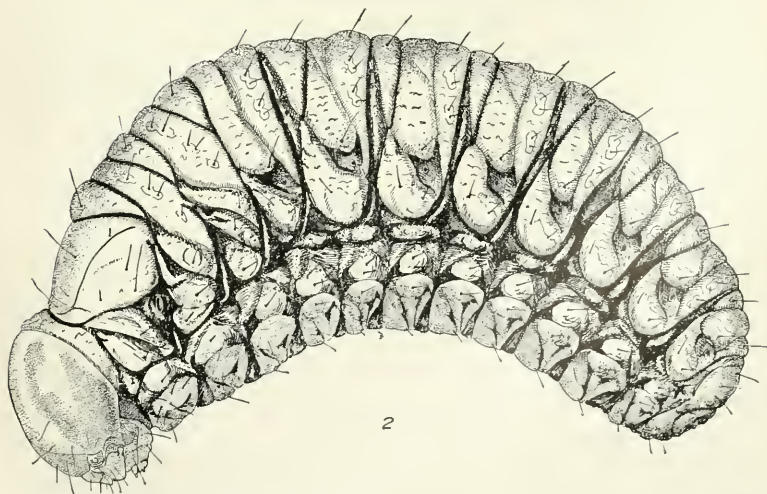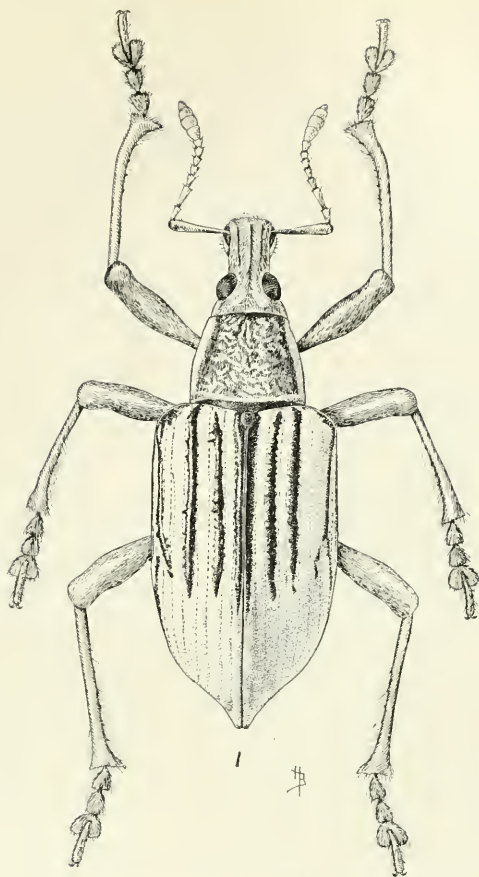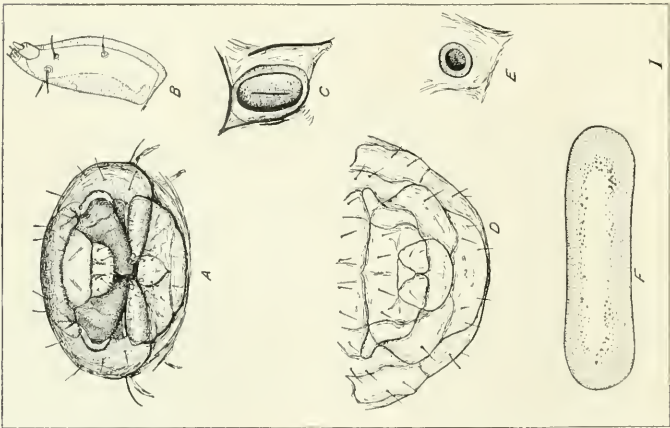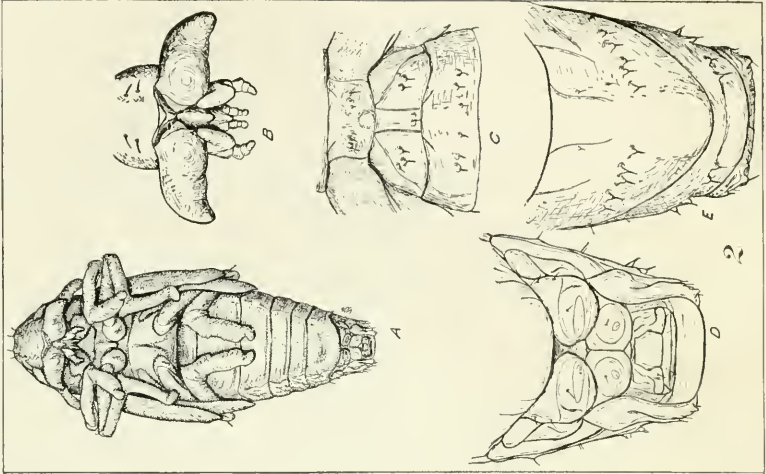
PLATE XXXVII

PLATE XXXVIII

Fig. 1.—*Diaprepes spengleri*, variety *festivus: A*, Face of larva, much enlarged; *B*, maxilla of larva, very greatly enlarged; *C*, thoracic spiracle of larva, very greatly enlarged; *D*, third abdominal spiracle of larva, very greatly enlarged; *E*, last abdominal segments, ventral view, much enlarged; *F*, egg, very greatly enlarged (natural size, 1 mm.).   Drawn by the author.

Fig. 2.—*Diaprepes spengleri*, variety *spengleri: A*, Pupa, ventral view (natural size, 19 mm.); *B*, mouth parts of pupa, very greatly enlarged; *C*, mesonotum, metanotum, and first abdominal segment, very greatly enlarged; *D*, ventral view of part of the seventh, and the eighth, ninth, and tenth segments, much enlarged; *E*, dorsal view of seventh, eighth, and ninth segments, much enlarged.   Drawn by the author.

A CONTRIBUTION TO THE LIFE HISTORY OF SPONGOSPORA SUBTERRANEA

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INTRODUCTION

The manner of infection of tubers of the potato (*Solanum tuberosum*) by *Spongospora subterranea* seems never to have been observed. Osborn (14)[1] has described and figured what he considers a single amœba in a potato cell, but as will be seen from the writer's descriptions this can not be regarded as a significant step in the process of infection. It has usually been assumed that each potato cell becomes parasitized by one, or at most only a very few, amœbæ in much the same way as suggested by Woronin (17) for cabbage cells attacked by *Plasmodiophora brassicae*.

Spongospora subterranea was first technically described by Wallroth (16) in 1842 as *Erysibe subterranea*. Although it seems to have been widely distributed in Europe, it received little attention until 1885, when Brunchorst (2), a Norwegian botanist, made a careful study of the disease. His observation of plasmodia in the potato cells led him to place it in the group of the Plasmodiophoraceae. It was his opinion that the organism could live saprophytically in the soil, and he states that it is a general belief among farmers that lime and excess moisture favor the disease.

In 1892 Von Lagerheim (9) reported *S. subterranea* as very generally distributed in Quito, South America, which is the native habitat of the potato and may also be the home of Spongospora.

Johnson (7) studied the germination of the spores and describes eight swarm spores coming from each cell of the spore ball. In stained preparations he thought that he was able to see approximately eight nuclei in some of the ungerminated spores. Johnson did not observe the way in which infection takes place, but suggests that the swarm spores find their way into the tuber through lenticels, sprouts, and wounds.

Massee (10) published an account of the disease in 1908. According to his description (11), which is very brief, the spores are uninucleate and on germination produce only one amœba. He did not observe infection, but thinks that the amœbæ may be able to infect new cells by passing through the pits present in the cell wall.

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[1] Reference is made by number to "Literature cited," p. 278.

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Osborn (14) was the first to make an extended cytological study of *S. subterranea* and described for the first time the formation of plasmodia from separate amœbæ within the host cell. He also discusses vegetative nuclear divisions, nuclear fusions, and supposed reduction divisions, but his description, as well as his figures, gives the impression that he has been rather free in interpreting what he observed. Osborn (14) saw the division of infected host cells and gives this as the only method by which the disease spreads in the tissues. He describes a single uninucleate amœba in a young potato cell as the earliest stage with which he is acquainted. This part of his description agrees with observations previously made by Horne (5).

In the early spring of 1913 Güssow (4) reports having received specimens of *S. subterranea* from Quebec and other provinces of Canada. This is the first account of the disease in North America. During 1913 powdery scab was found in one of our chief potato sections by Melhus (12), who reported it on the 1912 crop of northern Maine. This section of Maine joins the Province of New Brunswick, Canada. The presence of Spongospora in the United States raises the question as to the effect this introduced parasite will have on our potato industry in the various parts of the country. This paper deals primarily with certain fundamental facts relating to the life history of the organism and its parasitic relations to the potato, which, as indicated above, are very imperfectly understood. In the author's studies of the germination of the spore balls of *S. subterranea* it has been found that a large number of small plasmodia are produced by the fusion of the amœbæ on certain cultural media. These results, coupled with previous observations in microtome sections of very young sori, show unmistakably that infection takes place through invasion by a plasmodium.

INFECTION OF YOUNG TUBERS

During the summer of 1913, while stationed at a field laboratory at Caribou, Me., the writer was able to obtain abundant material of *S. subterranea*. Various stages of the disease on young tubers, were fixed in Flemming's solution. This material was embedded in paraffin in the usual way and has been the source of most of the sections from which this study was made. Material for the study of spore germination was obtained from the crops of both 1913 and 1914.

On August 20 some young potato tubers were brought into the laboratory showing brownish-colored blisters easily recognized as very early stages of *S. subterranea*. Others showed none of these blisters, but instead very small, inconspicuous, light-brown-colored circular spots. These spots were never more, and usually less, than $\frac{1}{4}$ mm. in diameter. On careful observation it could be seen that each of the tiny brownish spots was surrounded by a circular translucent area that varied from 1 to 2 mm. in diameter. In most cases its limits could easily be distinguished. The light, brownish spot at or very near the center of the translucent area was

not sharply limited, but gradually faded out along its edges. On the whole, these spots, each surrounded by a faintly grayish colored, translucent ring, presented much the appearance that might be obtained by injecting a small drop of water beneath the epidermis of a young tuber. A study of free-hand sections through some of these spots led to the conclusion that they were caused by a small, disk-shaped drop of light grayish colored material just beneath the epidermis. Some of these spots were fixed in Flemming's stronger solution and were labeled "halo stage of Spongospora," because when held in the sunlight the small brownish spot had the appearance of being surrounded by a halo. Some of this material was sectioned and stained along with other early stages of the disease before plasmodia had been observed in culture. Saprophytic plasmodia produced in culture media have been studied parallel with investigations of the microtome sections through the translucent areas.

Early and late stages of infection have been obtained, and many of the slides show with great clearness that the potato tissue is first invaded by the fungus in its plasmodium stage. The light-brown spot at the center of the translucent area above described is undoubtedly the point at which the plasmodium enters the skin of the tuber. The limits of the translucent area mark the distance to which the plasmodium has spread beneath the epidermis.

In stained sections it is possible to observe in detail the manner in which the potato tissue is attacked by the plasmodium. Some of the preparations show it passing down through and between the epidermal cells. Usually a considerable number of cells are killed at the point where the plasmodium enters. Once beneath the epidermis, it spreads out in all directions and forms a rather flat, disk-shaped mass, which separates the epidermis from the tissue beneath. In general, the potato cells in contact with the plasmodium are at once stimulated to abnormal growth and division, but some of them are killed as the plasmodium spreads out over the healthy tissue. In this way it comes to occupy a space between the uplifted epidermis and sound tissue beneath. This circular plasmodial mass is thicker at the point where it enters the epidermis than toward the edges. At first the lower surface of the disk-shaped mass is almost smooth. It comes in intimate contact with the cells on which it rests. Soon, however, a number of projections of pseudopodia begin to extend downward, push in between the cells of the sound tissue, and seem to crowd them apart. All of the cell walls that have been or are in contact with the plasmodium stain differently from those in healthy tissue, and such cell walls are often somewhat swollen, showing a special affinity for the orange stain of the triple combination. This indicates that the cellulose is being acted on and in some manner changed, but whether the change is accomplished through the secretion of an enzym the writer is unable to say. It is possible that

such changes might be the result of the direct action of the protoplasm of the plasmodium on the cellulose.

As the pseudopodia push down between the cells the walls of the latter seem to become somewhat gelatinous and the middle lamella is dissolved. These tapering plasmodial projections sometimes extend down into the sound tissue for a distance of five or six cell layers; often, however, they do not go deeper than two cell layers. They are irregular in shape, as might be expected from the way in which they crowd between the cells. Through these pseudopodia the plasmodium comes in contact with a large number of cells. The cell walls seem to become more and more softened and gelatinous as they thicken, and may even lose to a certain extent their original shape, becoming wavy. Plate XXXIX, figure 4, shows the characteristic way in which the plasmodium pushes down between the cells. Deeply stained globular bodies are also conspicuous in it, two of which are sometimes joined together. In certain portions of the plasmodium nuclei can be distinguished, but they are poorly fixed. Plate XL shows a small part of an infecting plasmodium. The cell containing the large nucleus is not yet infected, but the one to the right of this has been penetrated. In Plate XXXIX, figure 3, is shown a vertical section through the parenchyma of the potato and an infecting plasmodium which can be seen spreading out over the healthy tissue and forcing the cells apart. Certain cells have already become infected; many of them are beginning to enlarge. They are stimulated to abnormal growth even before penetration, which suggests that the stimulus may be due to a secretion from the plasmodium.

Through the softened cellulose walls the plasmodium sends small protoplasmic strands, which may for convenience be termed the "infecting pseudopodia." These usually pass into the cell through openings that are quite small, but occasionally the opening is rather large, as shown in Plate XXXIX, figure 5. In this figure is shown a potato cell that is being infected. The plasmodium has made an opening in the wall and is flowing through and apparently into the protoplasm of the cell. The nuclei of *S. subterranea* are well stained. The large nucleoli stain a bright red; the chromatin strands, blue. The host nucleus is also clearly shown. A large nucleolus and chromatin strands can be seen. In some manner which has not yet been determined the infecting pseudopodia become separated from the remainder of the plasmodium, and in this way the individual cells receive each a small portion of protoplasm from the plasmodium that is invading the tissue. The quantity of infecting material received by different cells is quite variable. The writer has not been able to decide what it is that determines the amount received by a given cell. Within the cell the little plasmodium often travels along the cell wall for a certain distance before actually entering the protoplasm of the host. Just how the plasmodium actually penetrates the protoplast of the host cell is a point that is not yet clear.

The stages by which it passes through the limiting membrane and into the protoplasm of the host have not been observed. This is partly due to the fact that most of the cells that are becoming infected or are newly infected are more or less plasmolyzed. Whether this plasmolysis is entirely due to fixation or whether it is in part due to the attack of the plasmodium is an open question. Numerous cases can be found in which the plasmodium has not yet gone far into the host protoplast. In such cases it is usually on that side of the cell from which it entered. Other stages can be found in which the plasmodium has just reached the host nucleus. In a later stage it completely or almost completely surrounds the host nucleus. This may properly be considered the last stage of infection.

The plasmodium is at no time obviously delimited from the protoplasm of the host, and there seem to be no membranes between the two. The meshes in the cytoplasm of the plasmodium are smaller than those in the cytoplasm of the host. The parasite takes the orange stain more intensely than the protoplasm of the host. It is therefore easy to distinguish the two, yet they blend into each other in such a way that it is impossible to determine sharply where the one begins and the other leaves off. Thus, the plasmodium in the host cell does not appear to be in a vacuole or to be separated from the host protoplasm by a membrane of any kind. The one seems to be somewhat miscible in the other, which is what might be expected if the surface tensions of the two plasms are equal. Czapek (3) has measured the surface tension of the protoplasm of widely separated groups and has found that in all cases it is approximately the same. This is an interesting point and deserves more detailed study.

The shape and general macroscopic appearance of the plasmodium as it attacks young potato tissue have already been described. Something should be said of its appearance in stained sections. The cytoplasm of the plasmodium is finely granular and shows a special affinity for the orange stain. Embedded in it in considerable abundance are the globular bodies that have already been mentioned. They stain very deeply with the gentian violet stain and often appear almost black. Various sizes are to be seen, which have been shown in Plate XXXIX, figures 3, 4, 5, 7, 8, and 9. They are also abundant in the photomicrographs shown in Plates XL, XLI, and XLII. The writer has not been able to discover what these bodies are or to observe that they have any specific structure. They are always present and very conspicuous in the infecting plasmodium and are carried with it into the potato cells. The considerable variation in their size and the intensity with which they take the gentian stain support the view that they may be encysted amœbæ that have been engulfed and are carried along. Starch grains, pieces of broken-down cell walls, and other foreign bodies are present in the infecting plasmodium. Nuclei can also be seen, but these are rather difficult

to stain. A few nuclei are shown in the infecting plasmodium in Plate XXXIX, figure 4. A nuclear membrane, nucleolus, and, in some cases, chromatin strands are to be seen. The nuclei stain much more readily after they have been carried into the host cells. Nuclear divisions have not been observed in the plasmodium before it enters the host cells.

In the way which has already been described the invading plasmodium infects a small pocket of tissue just beneath the epidermis. This varies from 1 to 2 mm. in diameter and from one to six cells in thickness. Its size determines the size of the powdery-scab sorus that is to result. If this small island of infected cells could be removed, it would leave a bowl-shaped cavity covered over by the epidermis.

Shortly after the cells become infected, they begin to grow very rapidly. Giant cells 5 or 10 times as large as the normal ones are soon to be seen. Instead of growing equally in all directions, the infected cells elongate, most of their growth being radially outward. This results in lifting the epidermis and finally in breaking through it. Plates XLI and XLII show the raised epidermis, while Plate XLIII, figure 1, gives a good idea of the appearance of the sorus after the epidermis has been ruptured. The torn edges turn back on all sides and give the appearance so characteristic of powdery-scab lesions. The individual cells at first become much enlarged. Their nuclei divide, sometimes mitotically, but much more often, it is believed, by direct division. In those cases where mitotic division of the nucleus occurs, a cell plate is formed and the cell becomes divided in the usual way. When the nuclei divide directly, the giant cells become multinucleate. Ultimately the giant cells are all cut up into smaller cells. Usually not more than five or six cells are produced by a single infected cell. Some of the giant cells and also some of the vertical rows of small cells that have resulted from the division of the large ones can be seen in Plates XLI and XLII. Plate XLI shows a vertical section through the edge of a young sorus. The epidermis has been slightly raised through the upward growth of infected cells. Just beneath the epidermis can be seen the dark intercellular spaces which were previously occupied by the infecting plasmodium. These spaces are now filled with débris left behind when the plasmodium entered the potato cells. The larger globules within the cells are the deeply stained bodies which are so common in the plasmodium. None of these bodies can be seen in uninfected cells. The smaller dark bodies are in most cases the nuclei of the parasite, and in some of the cells they can be seen clustered around the host nuclei. At this stage the host nuclei are spherical, and each contains a red-stained nucleolus. In this section most of the giant cells have been cut up into smaller ones, but two of them are still present. It can be seen that most of their growth has been radially outward. In Plate XLII can be seen a somewhat later stage than was shown in Plate XLI. Each host nucleus is embedded in a plasmodium. The intercellular spaces beneath the epidermis, which

were previously occupied by the infecting plasmodium, are quite clearly shown in this illustration.

The cells produced by divisions of the giant cells are all infected. They are approximately the size of the normal potato cells and seem to grow rather slowly. So far as has been observed, these cells rarely divide. Half a dozen, or even more, infected cells may result from one cell originally infected. The writer has never seen any indication that a plasmodium can pass from a growing infected cell into a healthy one.

Within the host cell the plasmodium is closely applied to the host nucleus, as shown in Plate XXXIX, figure 6. The host nucleus seems to be as thoroughly embedded in the plasmodium as are its own nuclei. Whether or not the fungus cytoplasm is in direct contact with the membrane of the host nucleus is difficult to determine, but, so far as appearance goes, this seems to be the case. It is interesting to see that the intercellular plasmodia do not kill the host cells, but merely stimulate them to increased growth and division. This relation indicates that *S. subterranea* is a rather highly specialized parasite.

Sometimes the host nucleus becomes much lobed and distorted; it often contains several nucleoli. The chromatin strands become abnormal in appearance or may even entirely disappear. In some instances, however, the host nucleus remains intact even after spore formation. It can then be seen embedded in the spore ball. The relation between the plasmodium and the host nucleus is an interesting subject, but the detailed description of the appearance of the diseased host cells will be left for some future time.

The plasmodium within the host cell is irregular in shape, as shown in Plate XXXIX, figure 6. The nuclei are rather evenly distributed and stain very readily. Each contains a large nucleolus. The nuclear membrane stands out clearly, and chromatin strands can be seen. The writer is of the opinion that these nuclei do not divide during the early stages of infection, but this point needs further study. Nuclear divisions in the plasmodium seem most common just after the giant host cell has divided into smaller ones. So far as has been observed, all the nuclei in a given plasmodium divide simultaneously. Mitotic divisions are the only kind that have been observed. The equatorial plate stage is shown in Plate XXXIX, figure 7. This is the stage most commonly met with in the preparations, but later stages are also abundant. Spindle fibers are clearly seen, but no astral rays or centrosomes have been observed. The writer agrees with Osborn (14) that nuclear division immediately precedes spore formation, but whether or not there are two successive divisions he is not prepared to say. In Plate XXXIX, figure 8, is shown a small portion of a plasmodium very highly magnified. A dense region can be seen around each nucleus. Appearances like this probably explain how Osborn (14) and others have been led to believe that the cells were infected by uninucleate amœbæ. It has generally been supposed that in

Spongospora, as well as in the other genera of the Plasmodiophoraceae, plasmodium formation takes place only a short time before spores are produced.

OBSERVATIONS ON THE SECONDARY INFECTION OF TISSUE AROUND THE OLD SORI

One of the most serious aspects of the powdery scab as it appears in the United States is the dry rot that often sets in around the sori during the fall and winter while the tubers are in storage. The dry rot is familiar to all who are well acquainted with the powdery scab in this country. Both Melhus (12) and Morse (13) have made mention of it in their bulletins on that disease.

The demonstration of the existence of a saprophytic plasmodial stage in the life history of *S. subterranea* as given below at once suggested the possibility that this might be responsible for the so-called dry rot around the old sori. Pustules showing the rot in various stages were fixed in Flemming's stronger solution and later sectioned and stained with the triple stain. Sections from this material plainly show plasmodia in the shrunken areas around the old sori. Here they can be seen feeding on the potato cells and killing them. Plate XXXIX, figure 9, shows a plasmodium pushing down between the walls of mature cells in tissue near an old sorus. It causes the walls to become slightly swollen in much the same way as was described for the infecting plasmodium in the tissue of the young tuber. The walls appear to be softened and more or less gelatinous. They are not swollen as much as in the case of the cells in young tubers. The plasmodium can be seen pushing into the cells through the softened cell walls. Sometimes the openings thus made are quite small, but often a large portion of the wall is broken down. Once through the wall, the plasmodium seems to distribute itself throughout the protoplasm of the host. The different stages of this process have not been carefully studied, and it is not known in this case again just how the plasmodium gets through the limiting membrane of the host cell. The cells are quickly killed, and the cytoplasm and nucleus disintegrate, leaving only the starch grains. The starch seems to be acted on very little by the plasmodium, which is rather surprising in view of its action on the cell walls. As soon as one cell is killed, the plasmodium passes on to the next, and in this way cell after cell is destroyed. In front of the plasmodium can be seen the healthy tissue, while behind it is left a disorganized mass of broken-down cell walls, starch grains, and other débris. It seems that the plasmodium has no harmful effect in advance of the cells actually attacked, as would be the case if some poisonous substance were secreted, but that the cells are killed by actually becoming engulfed in it. The line between healthy and diseased tissue is very sharp.

Although the dry rot usually extends in all directions from the old sorus, the plasmodium is generally to be found only on one side. Nevertheless, the broken-down cells throughout the dry-rot area show the path that it has taken, indicating that the plasmodium moves about as it feeds on the tissue around and beneath the old sorus. It is not common for the rot to extend very deep, but plasmodia may occasionally be found as much as 6 or 8 mm. beneath the surface of the tuber. They usually feed in the tissue immediately beneath the epidermis, and whether or not they may go deeper into the tuber after it has been planted is a question that needs investigation. When infected potatoes are used as seed, the mother tubers undoubtedly harbor the plasmodia during the growing season.

In sections through some of the sori that are just beginning to show the dry rot very young plasmodia can be seen. Many of the spore balls in the base of the old sorus show germination, each cell of a spore ball producing a single uninucleate amœba. This method of germination agrees with that observed when the spore balls are placed in culture media. Sometimes, however, the walls of an entire spore ball disintegrate, leaving the amœbæ in such close contact with each other that they seem to fuse and can not be distinguished as separate bodies. Thus, a single spore ball may give rise to a baby plasmodium, several of which coming together form much larger ones.

The plasmodia in these secondary infections have much the same appearance as those which infect the young tubers. In favorable sections nuclei and the characteristic deeply stained globular bodies can be seen. The nuclei are rather poorly fixed, but a nuclear membrane and nucleolus can be distinguished.

It is interesting to compare the effect of the plasmodium on the growing cells in the young tubers with its action on the mature cells in the tissue around the old sori. The growing cells are not killed, but are stimulated to increased growth and division; and it is this growth and proliferation of cells that produces the raised sorus. The mature cells, on the other hand, are quickly killed, the tissue is destroyed, and shrunken, discolored areas result. The sorus is somewhat definite as regards shape, size, and number of cells attacked. Although the dry-rot areas are usually not very large, there seems to be no limit to the number of cells that may be destroyed when secondary infection occurs. The writer is of the opinion that the dry rot may be considered a mild form of the canker stage of *S. subterranea*. The canker stage by which deep holes are eaten into the tuber is, in all probability, an especially virulent form of the plasmodial stage in secondary infections. It is frequently mentioned in the European literature and is considered a serious form of the disease.

Other types of dry rot following *S. subterranea* and associated with the presence of *Fusarium* spp., *Phoma* spp., and other wound parasites also occur.

OBSERVATIONS ON SPORE GERMINATION

The germination of the spores in the base of the old sorus has already been described. The writer has also studied their behavior in culture media. One of the first problems undertaken in this study of *S. subterranea* was that of spore germination. The spore balls were placed in a number of different substances, including distilled water, tap water, various sugar solutions, and potato infusions. The conclusion was soon reached that the spores germinate quite readily in media, but preparations favorable for demonstrating germination are not so easy to obtain. The demonstration of germination is, in fact, very difficult. The walls of the spores are so opaque that it is usually impossible to determine in unstained material whether or not a given cell has germinated. The amœbæ are quite small and hyaline. In liquid media they soon crawl away from the spore ball, and may easily be confused with protozoa.

The demonstration was finally accomplished by germinating the spores on agar media in Petri dishes. When a rather dry agar is used as the culture medium, the amœbæ remain, for a time at least, clustered around the mother spore ball. This gives an opportunity to observe the colonies produced by individual spore balls. When such material is fixed in Flemming's weaker solution, embedded in paraffin, sectioned and stained with the triple stain, it furnishes excellent opportunity for the study of germination. In this way permanent slides have been made which clearly show various stages of germination. Portions of germinating spore balls are shown in Plate XXXIX, figures 1 and 2. Figure 1 shows a small portion of a germinating spore ball from which some of the amœbæ are being set free. In one of the amœbæ shown in this illustration the nucleus can be clearly seen. The ungerminated spores are uninucleate. Figure 2 shows a small colony of amœbæ that have been set free through a partial disintegration of the old spore ball. The small deeply stained bodies are probably nucleoli; the nuclei can not be distinguished in these amœbæ.

In some cases the spore walls of the entire spore ball disintegrate, setting free as many amœbæ as there were cells in the spore ball. Generally, however, the amœbæ escape through openings in the walls of the individual spores, and the spore ball is left almost intact. Numerous spore balls showing nothing but empty walls can be seen in certain preparations. So far as has been observed, each spore contains only one nucleus and produces on germination a single, uninucleate amœba. The amœbæ are quite small, but under favorable conditions they grow rapidly and divide. They move about by means of pseudopodia. Cilia have not been observed.

A very good method for obtaining abundant germination is as follows: Mature spore balls taken from ordinary sori are dusted over the surface of a nutrient agar, such as Lima bean or potato agar in Petri dishes, just

before it hardens. They float on the surface of the medium and are held firmly in place when it hardens. The intimate contact thus obtained seems to give better germination than when the spore balls are dusted over the surface of the agar after it has hardened. Either method, however, will give good results.

In water or on agar containing no organic food material, such as sugars or proteids, germination takes place only after a considerable period of time. Under such conditions the spores may remain inactive for a month or longer, and even then a rather small percentage of them germinate, while on nutrient agar abundant germination is usually obtained. The spore balls in a given culture show considerable variation in the time required for germination. On a favorable medium many of the spore balls germinate within a few days, but in no case have all of the spore balls germinated, even when left on the agar for as long as two months. Why it is that the spores in certain of the spore balls fail to respond is a question that remains to be solved.

As the amœbæ leave the mother spore ball they crawl out over the surface of the agar. If the medium is allowed to become somewhat dry, they round up, produce a thick, rough wall, and while thus encysted are probably able to withstand various unfavorable conditions. The thick wall suggests that they would be quite resistant to desiccation, temperature variations, and toxic substances. They are generally uninucleate, but occasionally a binucleate cyst may be found. Under favorable conditions the cysts germinate. Through some means a hole is made in the thick wall and the amœba crawls out, and again encysts as soon as conditions become unfavorable. It seems that this process can be repeated an indefinite number of times, the cysts or resting spores furnishing a means by which the fungus may live over in the soil from year to year.

OBSERVATIONS ON PLASMODIA PRODUCED IN CULTURES OF GERMINATING SPORES

Mention has already been made of finding plasmodia in the cultures of germinating spore balls. The question that at once arises is whether these plasmodia are produced through the fusion of the amœbæ of *S. subterranea*. The only way in which this problem can be definitely and finally solved is through infection experiments. An effort has been made to infect young potato tubers growing in a greenhouse by placing one or more plasmodia on them, but this work has not yet yielded satisfactory results. The method followed was to remove the soil from young tubers without breaking them from the mother plant or otherwise injuring them. The tubers are then washed in water, and a small piece of agar covered with a plasmodium similar to that shown in Plate XLIII, figure 2, is placed on each. In this way the plasmodium is brought into direct contact with the skin of the tuber. The tubers are then covered with

soil and left for a week or more before they are again observed. In a number of cases in such cultures the plasmodium passed through the skin of the tuber and killed some of the cells beneath the epidermis, but no typical sori have been produced.

Although the infection experiments have not yet given satisfactory results, the writer is strongly of the opinion that the plasmodium which he has in culture belongs to *S. subterranea*. These plasmodia have been obtained more than 100 times from cultures of germinating spores. All of the plasmodia are alike in appearance, and many of them have been seen to engulf the amœbæ of *S. subterranea* as they crawl about over the agar. A considerable number of the plasmodia have been isolated and grown separately on Lima-bean agar. By making transfers about once a week they can be kept in an active growing condition. Some of them have been obtained in pure culture, but such cultures soon become abnormal and die. Some evidence has been gained that if either certain bacteria or fungi be added to the culture, the plasmodium flourishes. This is in agreement with observations made by Pinoy (15) on other slime molds.

Some of the plasmodia have been induced to crawl up on glass slides, where they can be fixed by dipping the slides in Flemming's weaker solution. When stained with the triple stain, numerous nuclei can be seen. Near the center of each nucleus is a rather conspicuous red-staining nucleolus. In size and staining reactions, as well as in their general appearance, these nuclei resemble very closely those in the plasmodia within the living potato cells.

If a culture is allowed to become dry, the plasmodium encysts, as is common among the Myxomycetes. In some instances, when a plasmodium is transferred to a fresh medium, the streaming motion stops. The plasmodium then breaks up, and certain portions of the mass crawl slowly away and soon produce fruiting bodies that closely resemble those of Polysphondylium. This mass as it crawls along builds a central stalk composed of irregular-shaped cells, much as has been described for members of the Dictyosteliaceae. The stalk may lie flat on the medium for a distance of several millimeters, but sooner or later it bends upward and serves as a sporophore. The stalk, which varies both in length and in breadth, is sometimes composed of a single layer of cells, but often is five or six cells in breadth. These sporophores frequently branch several times. The spores are born in much the same way as has been described by Brefeld (1) for the genus Dictyostelium. They are regularly cylindric and about twice as long as broad. On germinating they give rise to amœbæ, pseudoplasmodia, and, later, to fruiting bodies like those just described. No other kind of fruiting bodies have been observed. Although it was not to be expected that a fungus with a true plasmodial stage would give rise to a pseudoplasmodium, this, nevertheless, seems

to be the case. When portions of encysted plasmodia are transferred to fresh agar, the cysts germinate and give rise to a large number of amœbæ, which form pseudoplasmodia and fruiting bodies like those above described.

SUMMARY

(1) So far as known, the type of infection here described has never before been observed. Infection of growing potato tubers by *Spongospora subterranea* is accomplished not by separate amœbæ, as has previously been supposed, but through the action of a plasmodium which invades the tissue and infects a large number of cells at each point where it enters. The conception of a plasmodium invading healthy tissue, pushing down between the cells, and finally infecting them, is, it would seem, new to pathology. *S. subterranea* actually lives within the protoplasm of its host. In this respect it differs from most fungi and bacteria and offers an especially favorable opportunity for the study of the relations of host and parasite.

This account of the life history of *S. subterranea* raises many interesting questions regarding other members of the Plasmodiophoraceae. The manner in which infection takes place is unclear in the life history of all the members of this group. Do the amœbæ of *Plasmodiophora brassicae* produce plasmodia outside of the living cabbage cells? Are the cabbage cells attached by uninucleate amœbæ as was supposed by Woronin (17), or do they become infected in a manner similar to that above described for potato cells attacked by *S. subterranea?* The distribution of the diseased tissue in the roots of the cabbage suggests the latter method of infection.

(2) The cells in each little island of infected tissue are stimulated to abnormal growth and division.

(3) While the tubers are in storage the spores germinate in the base of the old sori and produce amœbæ which come together to form plasmodia that cause secondary infections.

(4) These plasmodia feed on the tissue around the old sori and cause a so-called dry rot, which is probably a mild form of the canker stage of the disease.

(5) The spores of *S. subterranea* germinate in culture media and each produces a single uninucleate amœba.

(6) When conditions become unfavorable the amœbæ encyst and go into a resting stage.

(7) The amœbæ seem to produce saprophytic plasmodia on culture media.

LITERATURE CITED

(1) BREFELD, Oscar.
    1872. Botanische Untersuchungen über Schimmelpilze. Heft 1, 64 p., 6 pl. Leipzig.
(2) BRUNCHORST, J.
    1887. Ueber eine sehr verbreitete Krankheit der Kartoffelknollen. *In* Bergens Mus. Aarster., 1886, p. 219–226, pl. 1.
(3) CZAPEK, Friedrich.
    1911. Über eine Methode zur direkten Bestimmung der Oberflächenspannung der Plasmahaut von Pflanzenzellen. 86 p., illus. Jena.
(4) GÜSSOW, H. T.
    1913. Powdery scab of potatoes (Spongospora subterranea (Wallr.) Johns.). *In* Phytopathology, v. 3, no. 1, p. 18–19, 1 fig., pl. 4.
(5) HORNE, A. S.
    1911. Preliminary note on Spongospora Solani, Brunch. *In* Ann. Bot., v. 25, no. 97, p. 272.
(6) JAHN, E.
    1907. Myxomycetenstudien. *In* Ber. Deut. Bot. Gesell., Bd. 25, Heft 1, p. 23–26.
(7) JOHNSON, Thomas.
    1907. Some injurious fungi found in Ireland. *In* Econ. Proc. Roy. Dublin Soc., v. 1, pt. 9, p. 345–370, pl. 32–35.
(8) ———
    1908. Spongospora solani, Brunch. (Corky scab.) *In* Econ. Proc. Roy. Dublin Soc., v. 1, pt. 12, p. 453–464, pl. 45.
(9) LAGERHEIM, N. G. von.
    1892. Remarks on the fungus of a potato scab. (Spongospora solani Brunch.) *In* Jour. Mycol., v. 7, no. 2, p. 103–104.
(10) [MASSEE, George].
    1908. "Corky scab" of potatoes. (Spongospora scabies Mass.) *In* Jour. Bd. Agr. [Gt. Brit.], v. 15, no. 8, p. 592–599, 1 pl.
(11) ———
    1910. Diseases of Cultivated Plants and Trees. 602 p., illus. London.
(12) MELHUS, I. E.
    1914. Powdery scab (Spongospora subterranea) of potatoes. U. S. Dept. Agr. Bul. 82, 16 p., 3 pl.
(13) MORSE, W. J.
    1914. Powdery scab of potatoes. Maine Agr. Exp. Sta. Bul. 227, p. 89–104, fig. 44–52.
(14) OSBORN, T. G. B.
    1911. Spongospora subterranea, (Wallroth) Johnson. *In* Ann. Bot., v. 25, no. 98, p. 327–341, pl. 27.
(15) PINOY, Ernest.
    1902. Nécessité de la présence d'une bactérie pour obtenir la culture de certains Myxomycètes. Note préliminaire. *In* Bul. Soc. Mycol. France, t. 18, p. 288–290.
(16) WALLROTH, F. W.
    1842. Der Knollenbrand der Kartoffel. *In* Linnaea, Bd. 16, Heft 3, p. 332.
(17) WORONIN, M. S.
    1878. Plasmodiophora Brassicae, Urheber der Kohlpflanzen-Hernie. *In* Jahrb. Wiss. Bot. [Pringsheim], Bd. 11, p. 548–574, pl. 29–34.

All figures were drawn with the aid of a camera-lucida and with Zeiss 2 mm. and 8 mm. objectives and oculars No. 4, 6, 8, and 12.

Fig. 1.—A small portion of a spore ball, showing the manner in which the spores germinate. $\times$ 1,500.

Fig. 2.—A portion of a spore ball and a small colony of amœbæ that have been set free by the disintegration of the spore walls. $\times$ 1,200.

Fig. 3.—A semidiagrammatic drawing of a section through a very young sorus, showing the infecting plasmodium as it pushes down between the cells. A few of the cells are already infected. Many of those in contact with the plasmodium are beginning to enlarge. The deep-staining globular bodies are distributed throughout the plasmodium. $\times$ 100.

Fig. 4.—An infecting plasmodium. The cells are being crowded apart as the plasmodium pushes down between them. The cell walls are becoming gelatinous and somewhat swollen, but are still intact. Deep-staining globular bodies and a few nuclei can be seen. $\times$ 1,000.

Fig. 5.—A potato cell becoming infected by a plasmodium of *S. subterranea*. The wall has been penetrated and the plasmodium is flowing into the cell. $\times$ 850.

Fig. 6.—A plasmodium closely applied to the host nucleus. The elongated host nucleus has a large and a small nucleolus, but is almost devoid of chromatin. $\times$ 1,200.

Fig. 7.—A small portion of a plasmodium. Simultaneous mitotic nuclear divisions are shown in metaphase. Spindle fibers are clearly seen, but astral rays and centrosomes are wanting. $\times$ 925.

Fig. 8.—Portion of a plasmodium within an infected cell, showing the dense cytoplasm around the nuclei and the clearer region between them. $\times$ 2,000.

Fig. 9.—A plasmodium pushing down between mature cells and causing secondary infection. The deep-staining globular bodies are present. $\times$ 1,000.
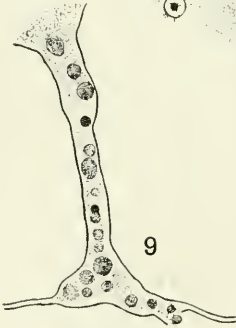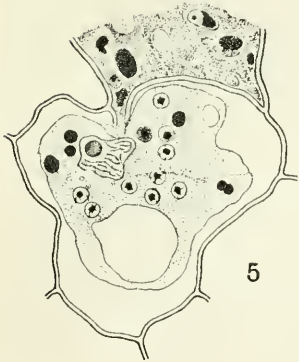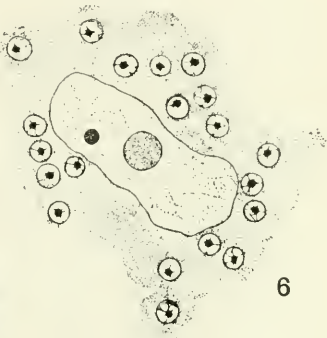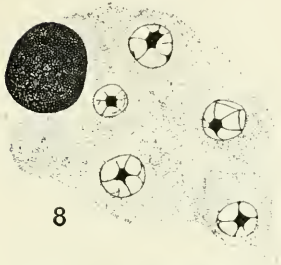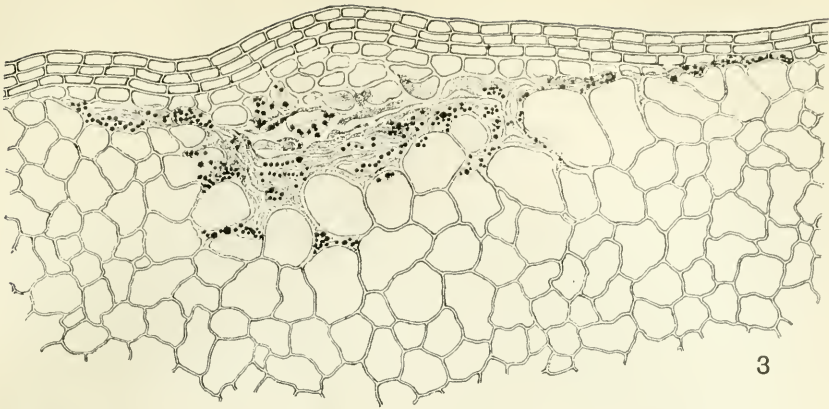
PLATE XL

Spongospora subterranea: Vertical section through a very young sorus, showing the plasmodium as it pushes down between the cells. × 1,000.

478

278 5'

Spongospora subterranea

Fig. 1.—A vertical section through a sorus soon after it has broken through the epidermis. × 50.

Fig. 2.—A living plasmodium obtained from a culture of germinating spore balls. × 5.
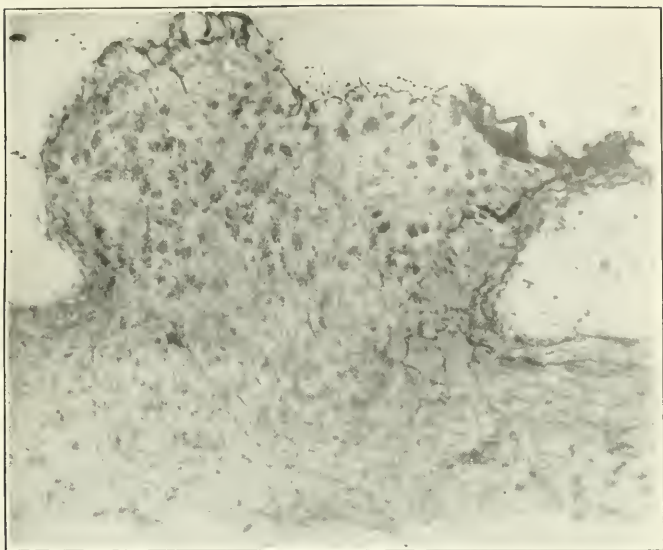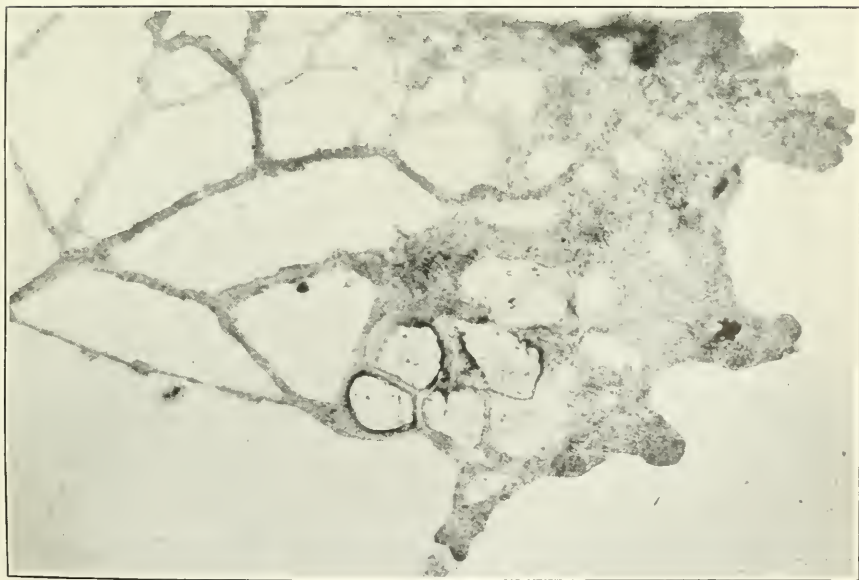
PLATE XLIII

1

2

RHEOSPORANGIUM APHANIDERMATUS, A NEW GENUS AND SPECIES OF FUNGUS PARASITIC ON SUGAR BEETS AND RADISHES

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INTRODUCTION

The fungus to be described in this paper was originally secured from damped-off seedlings of sugar beets (*Beta vulgaris*) which were grown in soil that had previously produced the black-root of the radish (*Raphanus sativus*). It was at first mistaken for *Aphanomyces laevis* De Bary and was so considered in a preliminary note.[1] The organism stands in a causal relation to both of the diseases mentioned, and recent trials have shown that it retains its virulence after a continuance of 30 months in artificial culture. The pathogenic relations of the organism were discussed in a recent paper,[2] but the fungus was not named or treated in its taxonomic relations. The present paper deals with the results of such further studies involving the morphology, cytology, and taxonomy of the organism as were found necessary to establish its identity and relationships and to make clear the various stages of its life history.

LIFE HISTORY AND GROSS MORPHOLOGY OF THE ORGANISM

In the general character of the disease produced in seedlings and in its appearance in cultures the organism resembles *Pythium debaryanum* so closely as to be readily confused with it, except in the asexual fruiting stage. The vegetative mycelium consists of nonseptate hyphæ which develop a profuse white aerial growth on suitable solid media, such as string-bean or oatmeal agar and certain cooked vegetables, of which the string bean is one of the most satisfactory. Oospores are formed in

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[1] Edson, H. A. Damping-off and root rot parasites of sugar beets. *In* Phytopathology, v. 3, no. 1, p. 76. 1913.

[2] Edson, H. A. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. *In* Jour. Agr. Research, v. 4, no. 2, p. 135–168, pl. 16–26. 1915.

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considerable numbers on string-bean agar. The normal life history, however, can be observed only under aquatic conditions. Cultures upon sugar-beet seedlings in water in Petri dishes where abundant aeration was obtained have been employed in the studies discussed in this paper.

A profuse, hyaline, nonseptate, branching mycelium with a finely granular internal structure develops (Pl. XLIV, fig. 12). The young hyphæ vary from 2.8 to 7.3μ in width, while portions of threads destined to function in reproduction may considerably exceed that diameter. Cultures 1 or 2 days old which have been well aerated at favorable (warm) temperatures exhibit remarkable protoplasmic streamings which, so far as they have been observed, are always directed toward the distal ends of the hyphæ. At such times the protoplasmic granules, or mitochondria, to which attention will be directed presently, may be seen to change their relative positions constantly and to exhibit a more or less independent motion. This protoplasmic streaming leads to an accumulation of material in the extremities of the threads, in consequence of which they become enlarged and more or less distorted according to the conditions. At length a partition wall is laid down to cut off the swollen portion from the rest of the mycelium; thus the first step in the process of fructification is accomplished (Pl. XLIV, fig. 7).

The body thus cut off merits special attention. It is similar in general appearance to the zoosporangia of various types which develop in the Saproligneaceae; but a study of its function shows that it differs distinctly from them, since it gives rise, not to zoospores, but to the body in which the zoospores are formed. While closely related in origin and appearance to the sporangium, it is correlated with important modifications in the process of zoogenesis which have not previously been described and constitutes a special organ with a new and sufficiently differentiated function to justify its designation by a distinctive name. The term "presporangium" is therefore applied to it. When fully formed, this body varies greatly in size and shape, depending upon the point where the cross wall is laid down. The presporangium may vary in length from less than 50 to more than 1,000μ. The width may scarcely exceed that of undifferentiated hyphæ, or it may increase to 20μ. Branching is present or absent according to the character of the segmented portion of the hypha before metamorphosis. After being cut off, these bodies increase in diameter, taking on a distinctly swollen and more or less distorted appearance. This step is accomplished apparently by taking up water which accumulates in vacuoles (Pl. XLIV, fig. 13, and Pl. XLVIII, fig. 1). The consequent development of turgor eventually becomes great enough to rupture the wall of the presporangium and permit the discharge of its contents, which emerge in an uncleaved condition (Pl. XLIV, fig. 6, 10). The rupture usually occurs at the extreme end of the hypha, though it sometimes takes place at the tip of one of

the side branches, which now present the appearance of swollen protuberances (Pl. XLIV, fig. 7).

The time at which the rupture of a given presporangium may be expected to occur can be predicted with reasonable accuracy some little time in advance by the appearance of the vacuoles, especially by one which develops at the tip so as to produce a relatively large hyaline area at the point where the break is to occur. This fact makes it easy to observe, with the oil- or water-immersion lens, the expulsion of the contents and its subsequent cleavage from the beginning, because in hanging-drop cultures the field within which it is to appear may be brought into focus before the phenomena begin to develop.

When the rupture occurs, there is at first a rush of protoplasm from the presporangium, soon to be modified to a steady flow of diminishing velocity. The entire protoplasmic contents flow out, leaving only the empty wall. The new body thus formed may be seen to be inclosed in a thin, membranous, almost invisible and very plastic wall, which is so flexible that the discharging mass takes on a spherical form as it is relieved from the pressure of the presporangium wall. During egress, therefore, the delivered portion presents the appearance of an enlarging sphere of protoplasm, while during the later part of the process the inclosed portion of the membranous wall may be seen advancing along the presporangium cavity, drawing out with it the last portions of the contents. This body, which is a zoosporangium, remains at the mouth of the presporangium wall during cleavage, although it does not appear to be attached to it by any visible means. Promptly following the egress of the sporangium, its cytoplasm cleaves into zoospores, which are liberated by the rupture of the sporangium wall.

The various steps in the process have been followed in the living material in hundreds of cases, as well as in the sections. In a typical instance observed in a hanging-drop culture under a water-immersion lens the liberation from the presporangium was completed at 11 a. m. Cleavage lines first appeared as indistinct grooves at 11.07. Short cilia were observed waving at the periphery at 11.09, and at 11.10 a rocking motion of the entire mass began. This continued uninterruptedly during the remaining time. The spores began to assume a definite outline at 11.13 and exhibited individual motion at 11.17. A large vacuole in each spore had become distinctly visible at 11.18. The spores began to change their relative positions quite freely at 11.25. They had assumed the normal adult shape at 11.26 and were swimming about with great activity within the sporangium. They escaped at 11.29 through a puncture produced in the membrane by the force of their impact upon it. In this instance 24 spores were counted. The number was commonly somewhat larger, approximating 50, although instances of as few as 4 were seen, and sporangia containing a number estimated to be 150 or

200 were quite frequent. In the majority of cases the enveloping membrane seemed to burst and then contract so as to allow the zoospores to escape almost simultaneously in all directions. It is usually impossible to discover any trace of the membrane after the spores have escaped. During the progress of cleavage the wall seems to function as a semipermeable membrane, since the distance between it and the cytoplasm increases somewhat and the size of the entire body increases by a few microns. The time which elapses between the egress from the presporangium and the liberation of the zoospores is usually about 30 minutes, though in some instances observed the entire process occupied only 17 minutes.

Under favorable conditions of observation at high magnification one may occasionally note an additional phenomenon, which appears to be related to the first appearance of cleavage furrows at the periphery of the contents of the sporangium. About the time the first indications of cilia are observable small, bubble-like protuberances suddenly puff out here and there and then instantly disappear as if they had burst. Thereafter cleavage indentations appear at the points of rupture, which continue to deepen until cleavage has been completed.

In a few instances the presporangium was observed to develop within the tissues of the host or in submerged agar in such a way as to permit of its reenforcement by the surrounding substance to such an extent that it was not ruptured, but remained intact. In such cases cleavage occurred within the presporangium, and the spores either escaped from the tip of the hypha for a swarm period or remained imprisoned within it, where they germinated. Instances were also observed in which germination occurred within the sporangium without liberation. Such cases, however, were seen only rarely and are not to be regarded as typical.

Following their escape, the zoospores swim about actively for a time, then come to rest, round up, increase in size to a diameter of about 11 or 12 μ while developing a large central vacuole, and send out germ tubes, generally two, which develop into typical vegetative mycelium from which zoospores are again produced. In the motile stage the zoospores are plano-convex, with a rather deep sinus on the flattened side. They always possess a single somewhat conspicuous vacuole and are biciliate. They have an average length of 12 μ and an average width of 7.5 μ.

Coincident with the later stages of zoospore production and following it, oospores are formed. The oogonium develops terminally as a spherical body from 22 to 27 μ in diameter. The antheridium, which develops either terminally or more generally in an intercalary position, is appressed to the oogonium. It is suborbicular, becoming cylindric to broadly clavate, averaging from 9 to 11 μ in width by 10 to 14 μ in length. The oospores are spherical and when mature have either a smooth or some-

what roughly undulated wall, averaging from 1.5 to 2.5μ in thickness. They have an average diameter of from 17 to 19μ (Pl. XLV, fig. 7).

Germination of the oospores was observed several times in the course of the studies. Water cultures in Petri dishes containing young sterilized sugar-beet seedlings were employed for growing the spores, which were produced literally by the thousand. After their development the cultures were allowed to dry out slowly without removing the covers from the dishes. When they had remained apparently air-dry for one month, sterile water was added to certain of them and they were observed for evidences of growth. In three out of five cultures zoospores were seen on the second day, but microscopic examination failed to demonstrate the presence of germinating oospores. While it hardly seems possible that the vegetative mycelium or asexual spores of the fungus would survive this drying on a glass plate for a month, there is reasonable ground for doubt as to the source of the growth. On June 15 similar cultures started in November and in January were tested for oospore germination in the same way. During the following three or four days, four out of five cultures yielded positive results which could be confirmed by microscopic examination. Of the many thousand spores present, however, only a very few germinated. These invariably put out germ tubes, which developed into vegetative mycelium bearing the characteristic asexual fruiting bodies.

When the available food supply has been consumed or the water has become sufficiently exhausted by gradual desiccation, aquatic cultures supplement by a further effort at preservation the fructifications already discussed. The cytoplasm which has not been used in spore formation collects in masses in different parts of the hyphæ, and walls itself off. These accumulations are most frequently found at the ends of threads, where they appear like small presporangia (Pl. XLV, fig. 6); but they occur also in other positions. They are more resistant to desiccation than ordinary hyphæ and doubtless serve under natural conditions to carry the organism through brief periods of drought.

DETAILED MORPHOLOGY OF THE ORGANISM

For the purpose of clearing up certain important details of the processes discussed in the preceding paragraphs, material from water cultures at various stages of the life history was killed in Flemming's weaker solution diluted with water, and embedded in paraffin, then sectioned and stained with Flemming's safranine, gentian violet, and orange G. For cilia the gentian alone was used. Camera-lucida drawings from this material are used to illustrate the following discussion.

The slides show that the nuclear divisions which precede oospore production occur only in the older portions of the hyphæ (Pl. XLIV, fig. 2, 3, 4, 5, 8, 9, 11). Following karyokinesis the daughter nuclei are carried

by the protoplasmic stream to the developing presporangium in which no divisions occur. The number of nuclei assembled before the organ is segmented off by a cross wall varies greatly. Presporangia containing as few as 4 were seen, while 200 or more were not uncommon. In the one illustrated 162 are shown (Pl. XLIV, fig. 7). The nucleus at this stage is spherical to oval and contains typically a single nucleolus located at one side, frequently protruding slightly from the body of the nucleus (Pl. XLVI, fig. 1, 8). In some cases, especially in the zoospore, the nucleus may contain two or even three nucleoli. The mitochondria, which are numerous in the vegetative hyphæ (Pl. XLVI, fig. 8), where, in certain stages at least, they are arranged so as to suggest a peripheral distribution, are still more abundant in the presporangia (Pl. XLVI, fig. 1), where they are evenly distributed throughout in great numbers. Following the development of a division wall to cut off the presporangium, numerous vacuoles develop within that body (Pl. XLVIII, fig. 1). These coalesce (Pl. XLIV, fig. 13) as they increase in size and develop the pressure which results in the rupture of the inclosing wall (Pl. XLIV, fig. 6, 10). Plate XLIV, figure 6, shows the condition just at the instant following rupture. The thin wall of the sporangium is seen covering the protruding cytoplasm, which is just beginning to escape. The sudden relief of tension is shown in a striking manner by the influence temporarily exerted on the form of the nuclei at the narrower portion of the body. Plate XLIV, figure 10, represents the process at a later stage. Here the rupture took place at the tip of one of the branches. At two places within the wall of the presporangium the membranous wall of the sporangium is seen receding along the cavity, while the flexibility of its structure may be judged from the spherical shape of the delivered portion.

Sections of the young zoosporangium show at first an entirely undifferentiated condition to be followed by the development of vacuoles and the migration of nuclei toward the periphery (Pl. XLV, fig. 5). The vacuoles coalesce, developing so as to form a relatively large irregular central cavity from which cleavage lines split outward. Meantime the nuclei arrange themselves at a uniform distance from the outer surface with their nucleoli turned toward the center of the mass (Pl. XLV, fig. 1). Cleavage furrows soon become apparent on the periphery so as to delineate the outlines of the future spores (Pl. XLV, fig. 4). Reference has already been made to the first appearance of these furrows on the exterior in living material. A single large vacuole develops near each of the nuclei (Pl. XLV, fig. 2, 4) and cilia appear in the indented areas between the future spores (Pl. XLIV, fig. 3). In this way a single row of zoospores is cut out at the periphery of the sporangium (Pl. XLV, fig. 2).

Cleavage usually progresses somewhat more rapidly in one side of the sporangium than in the other. It sometimes happens that the first

spores to mature succeed in rupturing the sporangium wall before the others have become mature. Such attached spores are often seen struggling about in the water until the completion of cleavage releases them from each other and they swim away. As already noted, cleavage may occur within the sporangium without its escape from the wall of the presporangium. In such cases the sporangium wall draws away from that of the presporangium so as to make it distinctly visible (Pl. XLIV, fig. 1).

The mature zoospores are uninucleate; hence, the number to develop in a given sporangium is predetermined by the number of nuclei contained within the presporangium. The number varies within wide limits; but, so far as accurate counts have been made, it has always been even. The zoospore nucleus is distinctly top-shaped, with the nucleolus at the broad end. The pointed end is directed toward the flattened side of the spore, where it ends in a blepharoplast, from which the cilia arise (Pl. XLV, fig. 3, and XLVI, fig. 6, 7, 9, 10, 11). The spores are plano-convex or slightly concavo-convex, with a sinus in the flattened side, which in the uncleaved sporangium is directed outward. The blepharoplast appears to come in contact with the spore membrane at the base of this sinus (Pl. XLVI, fig. 9, 10, 11).

It has already been pointed out that the cilia become visible in the living material before cleavage is complete. The sectioned material bears out this observation (Pl. XLV, fig. 3). When first observed in hanging-drop preparations, the cilia are quite short; but it may be seen that they elongate rapidly during the progress of cleavage so as to give the appearance of being pushed out slowly from within. The growth is sufficiently rapid almost to be seen at magnifications of 2,000 diameters—that is, one readily notes that they have increased in length in the course of a few seconds during which the attention has been fixed upon them. When mature, they are relatively long, but of unequal length. It is interesting to note that their combined length approximates twice the greater circumference of the spore, as is shown in Table I.

TABLE I.—*Relation of length of the cilia to the circumference of the spores (in microns)*

Length of shortest cilium.	Length of longest cilium.	Combined length of cilia.	Average length of cilia.	Greater circumference of spore.
19	31. 5	50. 5	25. 25	24
16	34	50	25	24
22	27	49	24. 5	25
16	33	49	24. 5	26
21. 5	27	48. 5	24. 25	24
21	25	46	23	27
14	29	43	21. 5	24
15	28	43	21. 5	21
10	31. 5	41. 5	20. 75	24
17	24	41	20. 5	22

In the absence of more definite information the evidence given in Table I might be used in support of the theory sometimes advanced that the cilia are of peripheral origin; but in the present instance at least it can be regarded only as an interesting correlation, since, as has already been pointed out, the cilia are put forth from the blepharoplast by a process of gradual elongation.

Each spore contains a single large central vacuole lying in contact with the nucleus and on the side toward the more pointed end of the spore (Pl. XLVI, fig. 4, 11).

The mitochondria are arranged in the zoospore at the periphery (Pl. XLVI, fig. 2). It is interesting to note that before cleavage furrows have pushed through the dividing protoplasm of the sporangium the mitochondria have already arranged themselves at what is to be the periphery of the future spore (Pl. XLV, fig. 3).

After coming to rest, the zoospore rounds up and undergoes certain changes preparatory to germination. Vacuoles develop (Pl. XLV, fig. 13), and karyokinetic nuclear division occurs (Pl. XLVI, fig. 15, 16, 17). One or usually two germ tubes develop (Pl. XLVI, fig. 3, 14, 17), forming a mycelium. The nuclei continue to divide within the spore and migrate into the mycelium, but divisions do not occur in the hyphæ until they have become mature (Pl. XLVI, fig. 3).

As the study of oogenesis is approached, it appears that both the antheridium and oogonium are multinucleate. The nuclei originate by karyokinesis in the parent hyphæ and migrate to the reproductive organs exactly as in the asexual stage. No divisions have been found to occur in either the antheridium or oogonium, although thousands have been sectioned and studied (Pl. XLV, fig. 8, and XLVIII, fig. 2, 3). After the requisite amount of material has been accumulated, the organs are cut off by cross walls. An eccentric cavity develops in the oogonium, so that the nuclei are arranged in a zone near the periphery (Pl. XLVIII, fig. 9). The vacuole then disappears; in its place there develops an area which takes the stain more densely than the surrounding cytoplasm, and a single nucleus comes to be within this area (Pl. XLVIII, fig. 8). In the meantime, the remaining egg nuclei undergo degeneration without division in the region where the wall of the oosphere is to appear (Pl. XLVII, fig. 2, 5, and XLVIII, fig. 5, 8). A receptive papilla forms on the antheridial side of the egg, and a passage way is opened from the antheridium through which a single nucleus and a considerable quantity of cytoplasm pass into the oosphere (Pl. XLVII, fig. 2, 5, 6, and XLVIII, fig. 8). The remaining antheridial nuclei degenerate (Pl. XLVII, fig. 2, 5, and XLVIII, fig. 8). The functional antheridial nucleus takes a position within the denser staining area at the center of the egg beside the egg nucleus and eventually fuses with it (Pl. XLVII, fig. 1, and XLVIII, fig. 5, 6, 7, 10).

There appears to be a certain amount of latitude as to the order in which some of the foregoing steps may occur. Sometimes the central

vacuole may persist till the degeneration of the supernumerary nuclei of the egg has reached an advanced stage (Pl. XLVII, fig. 2). Fertilization may be delayed till the degeneration of the nuclei is nearly complete (Pl. XLVII, fig. 5); or in exceptional cases it may occur even before the supernumerary nuclei have lost their nucleoli (Pl. XLVII, fig. 6). It is also apparent that the dense area at the center within which fusion occurs may arise before the degeneration of the nuclei (Pl. XLVII, fig. 6); or it may not appear till after fertilization has occurred (Pl. XLVII, fig. 2).

This body, which at once suggests a cœnocentrum, has been the subject of much speculation. Its presence in the species to be described is easily demonstrated. The functional nucleus of the egg takes a position within it where it is joined by the male nucleus, and the two fuse within it. Shortly after fusion it disappears, to be replaced by the lypoid material and stored food of the oospore. Critical study of the origin of the lypoid body, however, clearly demonstrates that the two are entirely distinct. The suggestion that the cœnocentrum-like body is the early stage in the formation of a food vacuole is not tenable in the case of this fungus, since that body is formed by the union of a large number of small lypoid granules which first develop outside the area in question, to coalesce later in the center of the spore (Pl. XLVIII, fig. 4, 6, 7, 10). On the other hand, it seems equally impossible to regard it as a true cœnocentrum originating from the centrosomes of the degenerating nuclei. The fact that these nuclei degenerate without previous division raises a certain, although not unsurmountable, element of doubt. The fact that it is not always present, even when degeneration has reached an advanced stage (Pl. XLVII, fig. 2), is significant, but the most conclusive proof comes from the fact that it may appear before degeneration has begun (Pl. XLVII, fig. 6). In Plate XLVII, figure 2, where the male nucleus has already entered the egg, the body in question is not present, and the egg nucleus remains at one side. It might be argued that the unusual position of the functional female nucleus at this stage is to be explained by the absence of the body and the consequent want of an attractive force to draw it to the center; in other words, that the mass under discussion is, in fact, a cœnocentrum. It seems more logical to the writer, however, to reason that the same force which causes the central vacuole to fill with denser protoplasm continues to act and that the accumulation of the denser material of the cœnocentrum-like body, the return of the female nucleus, and the approach of the male nucleus, as well as the subsequent accumulation of food material in the center of the spore, are but manifestations of its presence.

It has already been noted that in fertilization a considerable quantity of cytoplasm passes with the nucleus from the antheridium (Pl. XLVII, fig. 5). In preparations stained to show mitochondria it may be seen that this cytoplasm carries a mass of mitochondria which are more

closely clustered here than in the remaining contents of either the egg or the antheridium (Pl. XLVII, fig. 6). This dense clustering indicates that the presence of mitochondria in fertilization is not merely accidental and invites speculation as to their function. Their orientation at the periphery in the vegetative mycelium, and more especially in the zoospores, suggests that they may be responsive to light stimulus, and their accumulation in the presporangia and sex organs may indicate a nutritive function. The evident close philogenetic relationship of the fungus to algal forms perhaps supports the view that they are plastids, possibly degenerate chloroplasts. Their participation in fertilization raises a query as to whether they may not be charged with some part in the transmission of hereditary characters. A more novel and very interesting suggestion recently proposed to the author is that these bodies may be liquid crystals, and that, if this be true, a study of their origin and organization may lead to an understanding of the physical link which binds life to nonliving material; that in them we may discover, evolving from the inorganic and nonvital, that combination of physical and chemical properties with matter which characterizes life.

Following fertilization, food vacuoles appear in the cytoplasm (Pl. XLVIII, fig. 6, 7, 10) and then gather in a mass at the center (Pl. XLVII, fig. 3, and XLVIII, fig. 4). This crowds the fused nucleus from its position and leads to the development of a cytoplasmic zone about the central lypoid body (Pl. XLVI, fig. 12, and XLVII, fig. 3). In the meantime a thick spore wall has developed. There is at first a lighter zone between the cytoplasm and the wall (Pl. XLVI, fig. 19, and XLVII, fig. 3), but this gradually disappears, evidently by the shrinking and contraction of the wall (Pl. XLVI, fig. 12, 20).

The fused nucleus embedded in the cytoplasmic zone at first contains two nucleoli located at the poles (Pl. XLVII, fig. 3). These eventually disintegrate, but before doing so they migrate from the polar positions into the body of the nucleus (Pl. XLVI, fig. 5, *a, b, c,* 12, 20).

Another interesting phenomenon was observed in scores of cases—in the fused nucleus only and in material from but two lots, so that the work on this point can not be regarded as complete or as thoroughly established as is desirable. Owing to the pressure of other duties, however, the studies are not to be continued, and the observation is recorded here in the hope that it may be of value to other interested workers. During the progress of the changes of the nucleoli mentioned in the preceding paragraph, a single small body appears in contact with the nuclear membrane at one end (Pl. XLVI, fig. 5, *a*). Shortly following this, two bodies are present, one of which moves around to the opposite pole (Pl. XLVI, fig. 5, *b, c, d,* 19, 20). The position of the body suggests a centrosome.

Following the stages already discussed, there occurs a division of the nucleus (Pl. XLVII, fig. 7), giving rise to two nuclei which come to full

maturity (Pl. XLVI, fig. 18), promptly migrate to opposite sides of the cell, and undergo a second division, from which four nuclei arise (Pl. XLVII, fig. 4). Somewhat later a third division gives rise to eight nuclei. This is the greatest number observed in any of the material sectioned, but it is possible that additional divisions occur with the advancing maturity of the oospore.

The stage shown in Plate XLVII, figure 7, appears to be a reduction division, but it has not been possible to obtain the convincing evidence that would be afforded by chromosome counts. The fused nuclei are larger and differ in shape and in reaction to stain from the nuclei of other stages, while the two that result from the heterotypic division are similar in size and appearance to other nuclei. The interval between the first or heterotypic, and the second, or homotypic, division, while sufficiently long for the nuclei to become fully mature and to migrate to opposite sides of the cell, is relatively brief, so that the binucleate stage is seen much less frequently than the others.

It would be interesting to follow the history from the 8-nucleate stage of the oospore through germination, but the practical prosecution of such studies can be attempted wisely only after the conditions which induce germination are better worked out.

TAXONOMY OF THE FUNGUS

The characters of the fungus discussed in the preceding sections clearly place it in the Saprolegniaceae, but in none of the existing genera of that family. Among the systematic works dealing with the group that by Minden,[1] is the most recent. The system employed, which differs in some important respects from those of Schröter[2] and Fischer,[3] seems well conceived and logical and is so constructed as to provide readily a coordinate place for a new genus having the characters of the one to which the fungus under consideration is assigned. It will therefore be used in discussing the relationships of the organism.

Minden divides the Saprolegniaceae into two sections, according to the method in which the spores are liberated: Section A, in which all the zoospores of a sporangium escape through a common opening, and section B, in which they do not escape through a common opening. Section A, to which belongs the organism being treated, comprises subdivisions with diplanetic and monoplanetic spores. The diplanetic subdivision, with which we are especially concerned, consists of two groups. The first provides for Saprolegnia and Leptolegnia, where the zoospores are distributed for a swarm period immediately on liberation from the sporan-

[1] Minden, M. D. von. Pilze. *In* Kryptogamenflora der Mark Brandenburg, Bd. 5, Heft 3-4. 1911-12.
[2] Engler, Adolf, and Prantl, K. A. E. Die natürlichen Pflanzenfamilien . . . T. 1, Abt. 1, p. 96. Leipzig, 1897.
[3] Rabenhorst, Ludwig. Kryptogamen-Flora Deutschland, Oesterreich und der Schweiz. Aufl. 2, Bd. 1, Abt. 4, p. 326. Leipzig, 1892.

gium. They then come to rest, undergo metamorphosis, and emerge as more or less bean-shaped, biciliated zoospores for a second and more prolonged period of motility. This group presents a pronounced condition of diplanetism.

The second group, on the other hand, comprises Achlya and Aphanomyces, forms that present a condition of what may be called "reduced diplanetism." Here the first swarm period is reduced to a simple migration from the sporangium. It occurs after the spores are cleaved and is followed by metamorphosis from which the zoospores emerge, as in the first group, for a prolonged period of motility.

The fungus under consideration seems to present a third and hitherto undescribed type of diplanetism, in which the first motile period consists in the migration of the entire uncleaved sporangium and its contents from the presporangium. This type of egress is new. In all related forms previously described the spores are differentiated before migration. The distinction seems sufficiently important to justify its recognition as of generic rank. The uncleaved protoplasm rather than the differentiated spore migrates. The process of metamorphosis is eliminated and the spores that arise from cleavage have the form characteristic of the second motile period in the other genera mentioned.

The tendency in the series outlined has been toward monoplanetism, but it hardly seems probable that such a condition has arisen in this manner, since Pythiopsis, the monoplanetic genus of the subdivision, has eliminated metamorphosis and the second motile period rather than the first, giving the entire interval of locomotion to its more or less egg-shaped spores, the form type characteristic of the first motile period of genera like Saprolegnia.

It will be noted that for taxonomic consideration the body previously termed a "presporangium" has been regarded as closely analogous to a sporangium. Some may prefer to discard the prefix and apply the term "sporangium" to the portion of the hypha cut off for purposes of spore production, giving another name to the thin-walled organ in which the spores arise. It has seemed to the author, however, that the name "sporangium" should be applied to the organ in which the spores are differentiated, and that the term "presporangium," while distinctive, is at the same time clear and accurate, conveying a true idea of the function of the body to which it has been applied.

In selecting a name for the fungus an effort has been made to choose one which is descriptive of some distinctive character. From the fact that the sporangium flows out from the presporangium and that its wall is so delicate as to be almost invisible, the name "*Rheosporangium aphanidermatus*" has been chosen. Its technical description is as follows:

RHEOSPORANGIUM, new genus.

Mycelium aerial or aquatic, well-developed, nonseptate, branched. Reproduction by zoospores under aquatic conditions and by oospores. Terminal, enlarged, more or less distorted mycelial-like prezoosporangia cut off from the ends of hyphæ. Sporangia thin-walled, normally escaping from the presporangium through a terminal rupture, cleaving into zoospores.

Rheosporangium aphanidermatus, n. sp.

Vegetative mycelium white, in water hyaline, nonseptate except in fructification, branched, finely granular, frequently exhibiting pronounced protoplasmic streaming. Young hyphæ varying in width from 2.8 to 7.3μ, averaging from 4 to 6μ, but frequently becoming wider as fructification approaches. Presporangia developing by the enlargement of terminal portions of hyphæ, unbranched or irregularly clavately to normally branched, length varying from less than 50 to more than 1,000μ, width from 4 to 20μ, distal portion usually unbranched and tapering to a rounded end. Sporangia with nearly invisible, flexible, membranous walls escaping from the distal end of the presporangia or, rarely, from that of one of the branches, becoming spherical on release, varying in diameter according to the presporangia, at once cleaving into zoospores. Zoospores escaping by the rupture of the sporangia, plano-convex, with a single central vacuole, and on the flattened side a sinus, from the bottom of which the two cilia of unequal length arise. Average size, 12 by 7.5μ. Oogonia terminal, spherical, 22 to 27μ in diameter. Antheridia terminal or intercalary, suborbicular, becoming cylindric or broadly clavate, average dimensions, 9 to 11 by 10 to 14μ. Oospores single, smooth or contoured, average diameter, 17 to 19μ.

The following illustrations are reproduced from camera-lucida drawings of fixed and stained material, except when otherwise stated.

Rheosporangium aphanidermatus:

Fig. 1.—Cleavage of the sporangium into zoospores within the wall of the presporangium. ×2,000.

Figs. 2, 3, 4, 5, 8, 9, 11.—Nuclear divisions in the mycelium. Fig. 9, ×2,000; others, ×3,000.

Fig. 6.—Portion of presporangium showing rupture at tip and the initial stage of sporangium egress. ×2,000. See also figure 10.

Fig. 7.—Maturing presporangium. ×250.

Fig. 10.—Later stage of sporangium egress from a branch of the presporangium. ×2,000. See also figure 6.

Fig. 12.—Vegetative mycelium; living. ×500.

Fig. 13.—Section of nearly mature presporangium, showing development of large vacuoles. ×2,000. See also Plate XLVIII, figure 1.

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Rheosporangium aphanidermatus:

Fig. 1.—Section of presporangium showing nuclei and mitochondria.   ×2,000.

Fig. 2.—Section of zoospore showing mitochondria.   ×2,000.

Fig. 3.—Advanced stage of zoospore germination.   ×2,000.

Fig. 4.—Section of zoospore showing position of central vacuole and nucleus. ×2,000.   See also figure 11.

Fig. 5.—Stages in the preparation of the fused nucleus of the oospore for division. ×2,000.

Fig. 6.—Zoospore showing position of sinus in side.   ×2,000.

Fig. 7.—Section through zoospore showing blepharoplast and sinus.   ×2,000.

Fig. 8.—Mycelium showing nucleus and mitochondria.   ×2,000.

Figs. 9, 10.—Sections of zoospores showing blepharoplast and attachment of cilia. ×2,000.

Fig. 11.—Zoospore showing orientation of the various structures.   ×2,000.

Fig. 12.—Maturing oospore showing fused nucleus, large central food body, and the wall, which is unusually deeply contoured.   ×2,000.

Figs. 13, 14, 15, 16, 17.—Stages in zoospore germination.   ×2,000.   See also figure 3.

Fig. 18.—Section of maturing oospore.   First division of fused nucleus completed. ×2,000.

Figs. 19, 20.—Maturing oospores showing usual type of wall and the fused nucleus in preparation for division.   ×2,000.   See also figures 5 and 12.
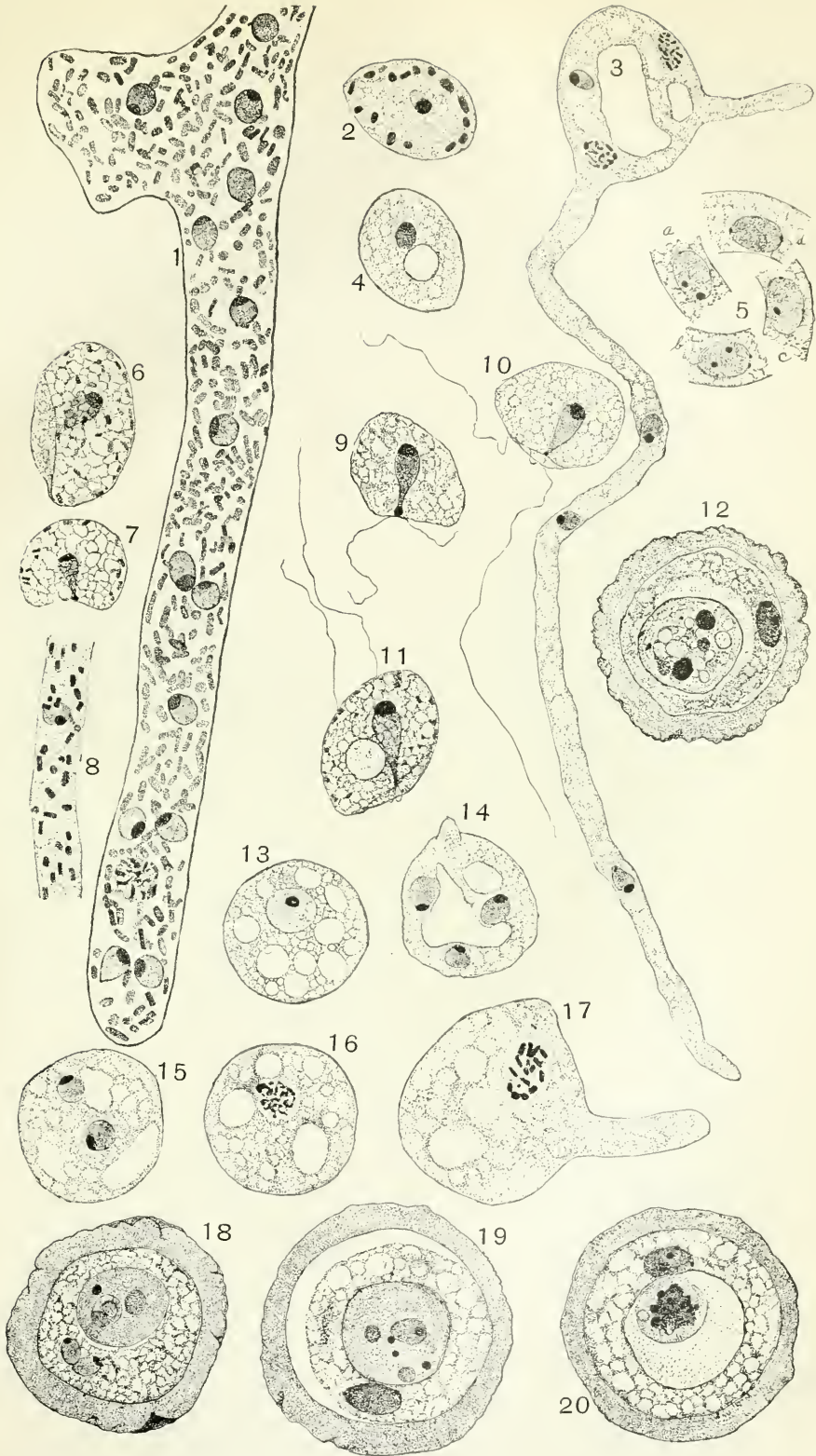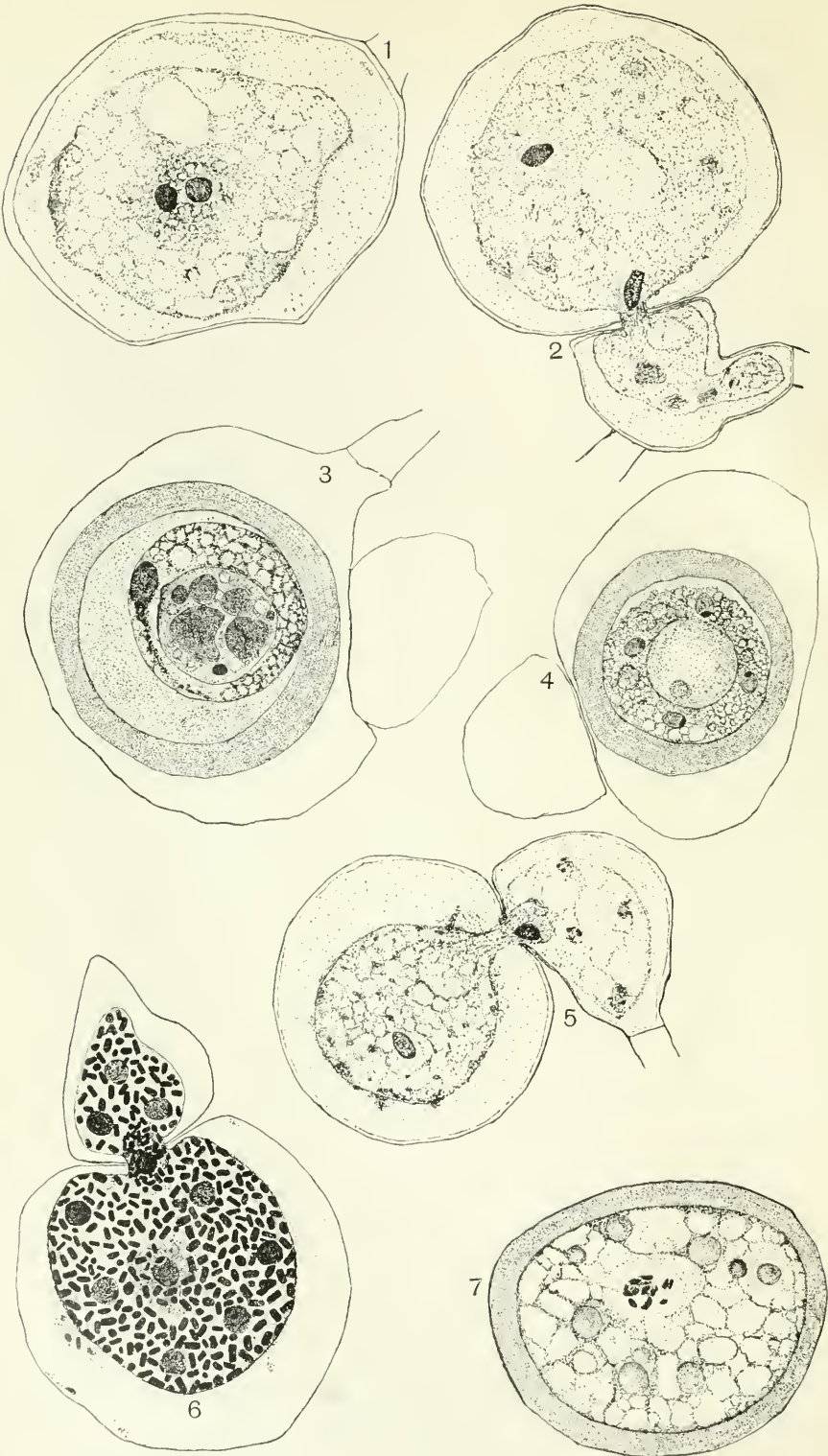
PLATE XLVII

Rheosporangium aphanidermatus:

Fig. 1.—Fertilized egg showing the two functional nuclei nearing juxtaposition. ×2,000.

Fig. 2.—Fertilization taking place before the central vacuole of the egg has entirely disappeared. Disintegration of supernumerary nuclei well advanced. ×2,000.

Fig. 3.—Maturing oospore showing the large central food body and the fused nucleus with two polar nucleoli. ×2,000.

Fig. 4.—Oospore after the second nuclear division. ×2,000.

Fig. 5.—Fertilization: Cytoplasm and functional nucleus passing from antheridium to oosphere. ×2,000.

Fig. 6.—Fertilization occurring before degeneration of the supernumerary nuclei. Mitochondria shown passing into the oosphere with the nucleus. ×2,000.

Fig. 7.—First division of the fused nucleus in the oospore. The central food body, which lies immediately beneath the nucleus, is not shown. ×2,000.

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PLATE XLVIII

Rheosporangium aphanidermatus:

Fig. 1.—Section of presporangium showing intermediate stage of vacuolization. ×2,000. See also Plate XLIV, figure 13.

Fig. 2, 3.—Developing oogonia and antheridia. ×2,000. See also Plate XLV, figure 8.

Fig. 4.—Oospore after nuclear fusion showing accumulation of food material at the center. ×2,000. See figures 6, 7, and 10 for earlier stages of food body.

Fig. 5.—Fertilized egg showing functional nuclei within the deeper stained central portion. ×2,000.

Fig. 6.—Fertilized egg showing functional nuclei in juxtaposition within central mass. Food bodies (decolorized) appear as vacuoles in the cytoplasm. Note the thickness of the wall, unusual at this stage. ×2,000. See also figure 10.

Fig. 7.—Fertilized egg showing nucleus after fusion within the deeper stained central portion and position of decolorized food bodies. ×2,000.

Fig. 8.—Typical fertilization: Functional egg nucleus in deeply staining center of oosphere; others degenerating at the periphery. Functional antheridial nucleus entering the oosphere; others degenerating in the antheridium. ×2,000.

Fig. 9.—Young oogonium and antheridium showing the central vacuole and the peripheral arrangement of the nuclei. ×2,000.

Fig. 10.—Fertilized egg: Oospore wall formed but not thickened, functional nuclei in juxtaposition within central mass, and food bodies in surrounding cytoplasm. ×2,000. See also figure 6.
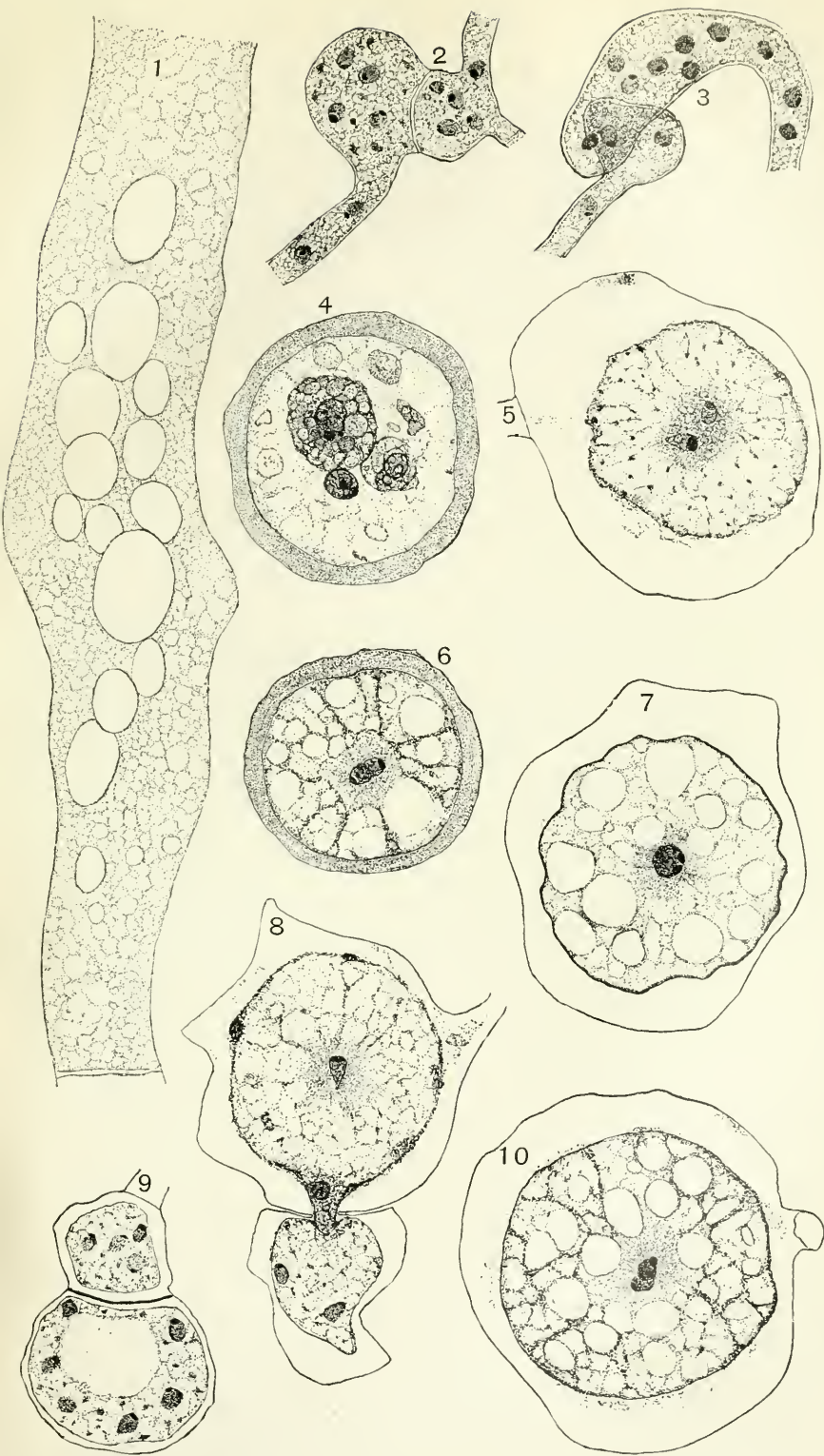
HEREDITY OF COLOR IN PHLOX DRUMMONDII [1]

By Arthur W. Gilbert,

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INTRODUCTION

Workers in the field of heredity, stimulated by Gregor Mendel's classical experiments, are attempting to prove or disprove the hypothesis, which is now quite generally accepted, that plants and animals are composed of distinctly heritable units, now called "unit characters." After the presence of these units has been demonstrated, the next problem is to determine what these units are in different plants and animals, and their exact mode of inheritance.

These experiments were planned to solve these problems with *Phlox drummondii.*

METHODS OF PROCEDURE

This plant was chosen because its flowers have a wide range of colors, it is easy to grow both in the greenhouse and out of doors, and crossing is not difficult.

Commercial seed was purchased and the different varieties grown and self-fertilized for three years, so as to be sure of pure types. The varieties used in these experiments were found to breed true for three years and are assumed to be pure.

The crossing was done in the ordinary way, great care being exercised at all times to prevent the admission of foreign pollen. All flowers were carefully bagged with small oiled bags, which were tied as tightly around the stem as the growth of the plant would permit. The parents were self-fertilized each year and grown alongside of the F_1 and F_2 hybrids.

Notes were carefully made of the color of the flowers, according to the nomenclature in Répertóire de Couleurs.[2] Inasmuch as the color fades rapidly in intense sunlight, the descriptions were made soon after the flower had first opened. These colors are described in the tables by naming the number of the page and the shade which corresponds nearest to it.

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[1] Paper No. 53, Department of Plant Breeding, Cornell University, Ithaca, N. Y.

[2] The following color book was used as a standard basis of comparison of the colors: Société Francaise des Chrysanthémistes. Répertoire de Couleurs. 82 p., illus., 3 pl. (2 col.), and 365 col. pl. in 2 portfolios. Paris, 1905.

Four varieties of phlox are employed in these experiments: Eclipse, Large Yellow, Coccinea, and Carnea. The Carnea variety was used as the male parent in all of the crosses.

Flowers that were practically colorless were recorded as "white" in the tables, but, as the color plates show, a very slight amount of color was present in many of them. They are probably not pure albinos.

THE NATURE OF COLOR

The colors of plants are due to constituents which are either colored themselves or act upon other substances to produce color in them. All of the cells of the plant contain these substances, with the possible exception of meristematic or rapidly growing tissue. Plant pigments may be divided into two classes with reference to their location in the cell: Chromoplast colors and cell-sap colors. The first class includes green, usually yellow and orange, and occasionally red; the second class, mostly red, blue, and violet.

Buscalioni and Traverso distinguish the following classes:

1. Green (chloroplasts).
2. Yellow and orange (chromoplasts).
3. White (colorless, made white by air in intercellular spaces).
4. Red.
5. Violet and lilac.
6. Blue (4 to 6 anthocyan pigments in solution).
7. Brown (tannin probably concerned).

Various other colors are supposed to be due to the mixing or modification of the pigments referred to. The black spots and stripes on the flowers of the broad bean, for instance, are evidently due to violet pigment, since the stripes at first are violet.

The yellow color of flowers is due in most cases to chromoplasts containing yellow anthoxanthin; but, rarely, yellow is a cell-sap color, as, for example, in *Mirabilis longiflora* and the yellow parts of a white dahlia. In the latter case there is a transition to red cell sap that establishes the close relationship of these two colors.

In yellow beets, also, there is yellow cell sap, probably closely related to the red sap color of the beet.

The yellow-brown colors found in seeds and fruits especially are considered to be largely due to, tannin, which is itself colorless but readily produces color through the action of carbon dioxid.

Reds are usually cell-sap colors. Chrome reds and brick reds are exceptions. The tomato and the carrot, for instance, have red chromoplasts.

The blue and the purple color substances in flowers are dissolved in the cell sap and are distinguished for the most part from the plastid colors by being insoluble in ether, xylol, benzol, chloroform, carbon disulphid, and similar solvents, but are soluble in water or alcohol.

PLASTID COLOR SUBSTANCES

There are found in most plant cells lying in the cytoplasm outside of the nucleus small bodies called "chromatophores." In embryonic cells and growing points these chromatophores are colorless and highly refractive, and this condition may be retained until the cells reach maturity. Ordinarily, however, these colorless chromatophores attain a further development as chloroplasts, leucoplasts, or chromoplasts.

CHLOROPLASTS

In parts of the plant which are exposed to the light, the chromatophores usually develop into chlorophyll bodies of flattened ellipsoidal shape and are scattered in numbers in the parietal cytoplasm of the cells. These granules contain two pigments, green and yellow, the former predominating often to the complete exclusion of the latter. The yellow pigments of the chloroplasts are collectively termed "xanthophyll." The green and yellow pigments may be separated from each other, and each can be readily seen by placing green leaves, which have been previously boiled in water, in alcohol and adding benzol. When this solution is shaken and then allowed to stand, the benzol will rise to the surface as a green solution, leaving the alcohol yellow.

CHROMOPLASTS

The chromoplasts of most flowers and fruits arise either directly from the rudiments of colorless chromatophores or from previously formed chloroplasts. The color of the chromoplasts varies from yellow to red, according to the predominance of yellow xanthophyll or orange-red carotin. The name "carotin" has been derived from the carrot (*Daucus carota*), in the roots of which it is particularly abundant. Carotin is practically identical with the so-called "chrysophyll" found in the chloroplasts.

It will be seen that there is a very close relationship between the chloroplasts and chromoplasts and the green and yellow colors found in them. In general, however, it may be said that the yellow color in certain roots, flowers, and fruits is due to the yellow pigments of the chromatophores.

CELL-SAP COLOR SUBSTANCES

During the process of metabolism the plant cell manufactures other color substances which are not combined with the protoplasm, but which are contained in the cell sap, or liquid of the cell. These substances, unlike other plastid colors, are insoluble in xylol, ether, or similar solvents, but are soluble in water and alcohol, which afford a means of separating them from the plastid colors. These cell-sap pigments may

occur in cells free from plastids or in the vacuoles of cells containing plastids, but not associated with them as a part of the organized body or plastid. These pigments have one property in common with the chromophyll substances—i. e., with alkalies, potassium cyanid, and sodium phosphate they assume some shade of green. They are distinguished, however, by the fact that the colors are markedly affected by acids and alkalies and by iron salts. The fact that these substances are so sensitive to reagents probably accounts for the various shades and tints characteristic not only of flowers but of leaves as well. Kraemer has observed in the germinating kernels of black Mexican sweet corn that even in contiguous cells the constituents associated with the dye vary to such an extent that the pigment in one cell is colored reddish, in another bluish green, and in another purplish.

COLOR HEREDITY

It will be seen that the nature of color is very complex, and consequently its heredity is equally so. Mendel's studies of peas and many other similar plants seem now comparatively simple, because they deal with characters which are easily distinguishable with little reaction of one upon the other. Each unit was found to be separately heritable and could quite easily be traced from generation to generation. Not so with colors, the units of which are obscure; being chemical in nature, reactions of various sorts occur, making experimentation difficult.

RESULTS OF CROSSING

SERIES I

POLLEN PARENT.—A pale rosy-pink variety known commercially as Carnea. The color corresponds to shade 1 on page 129 of the Repertoire de Couleurs, designated hereafter as "shade 129–1." This variety has a white eye—that is, the center of the flower is white and arranged as a distinct pattern (Pl. C).

SEED PARENT.—A violet-purple variety (192–3) known in commerce as Eclipse. This variety had a dark eye—that is, the center of the flower had a denser, deeper color than the remainder of the flower, but without any particular pattern.

FIRST AND SECOND GENERATION HYBRIDS.—The F_1 hybrids were unlike either parent. They were Tyrian rose (155–4), with a dark eye. The color of the F_1 hybrids immediately suggested the presence in the parents of complementary color factors, which were united to produce something different from either parent.

All plants having similar colors were placed together in groups and the numbers recorded. Table I gives the results of the F_2 hybrids grouped as accurately as possible in this way.

TABLE I.—*Color of the progeny of two varieties of Phlox drummondii, the Eclipse and the Carnea*

Parent or progeny.	Phenotypic formula.	Phenotype.	Field count.	Calculated.	Ratio.
Seed parent: *Phlox drummondii*, var. Eclipse.	EBrI.........	Violet-purple (192–3), dark eye.	....	....	....
Pollen parent: *Phlox drummondii*, var. Carnea.	ebRi..........	Pale rosy pink (129–1), white eye.	....	....	....
F₁ generation..............	EeBbRrIi.....	Tyrian rose (155–4), dark eye..	....	....	....
F₂ generation: Alternative factors—					
EEBBRRII..	Reddish violet (180–4), dark eye (Pl. C, fig. *A*).	204	171. 81	81
EEBBRRii...	Bright violet (198–4), dark eye (Pl. C, fig. *B*).	16	57. 23	27
EEBBrrII....	Bright violet-purple (190–3), dark eye (Pl. C, fig. *C*).	41	57. 23	27
EEBBrrii.....	Dauphin's blue (203–2), dark eye (Pl. C, fig. *D*).	39	19. 09	9
EEbbRRII...	Crimson carmine (159–3), dark eye (Pl. C, fig. *E*).	58	57. 23	27
EEbbRRii....	Pale lilac-rose (130–1), dark eye (Pl. C, fig. *F*).	12	19. 09	9
EEbbrrII.....	White, pigmented (pink) eye (Pl. C, fig. *G*).	1	19. 09	9
EEbbrrii......	White (Pl. C, fig. *H*)..........	(a)	(a)	(a)
eeBBRRII....	Magenta (182–3), white eye (Pl. C, fig. *I*).	71	57. 23	27
eeBBRRii....	Pale light lilac (187–1), white eye (Pl. C, fig. *J*).	6	19. 09	9
eeBBrrII...... eeBBrrii......	Pale purple, white eye (Pl. C, fig. *K*).	17	24. 45	12
eebbRRII.....	Tyrian rose (155–3), white eye (Pl. C, fig. *L*).	10	19. 09	9
eebbRRii.....	Pale lilac-rose (130–1), white eye (Pl. C, fig. *M*).	65	6. 36	3
eebbrrII...... eebbrrii.......	White (Pl. C, fig. *H*)..........	3	14. 84	7
Total..............	....	....	543	....	256

a See explanation of white below.

EXPLANATION OF SYMBOLS AND FACTORS IN TABLE I

The results from the F₂ hybrids suggest that the following factors are present in the parents. These factors have been represented by symbols as indicated below.

"E." A factor for dark eye color producing a denser coloration at the center of the flower, or eye. E does not act in the absence of the color factors B, R, or I (Pl. C, fig. *G*), but is effective if any one of them is present.

"e." The absence of an eye factor produces a white eye. The latter, unlike the dark eye, seems to have a distinct pattern (Pl. C, D, E).

"B." Presence of blue pigment. This is not a pure blue, but contains red, producing purple. The blue and red do not become dissociated, but are inherited together throughout the series. This red seems to bear no relationship to the red (R) brought in by the pollen parent, Carnea.

"b." Absence of blue.

"R." Presence of red. This is a distinct red factor which is inherited separately from all others. The absence of the factors for color, B and R, produces white.

"r." Absence of the red brought in by the pollen parent.

"I." An intensifying factor which determines the degree of pigmentation. This seems to affect the red only (Pl. C, fig. *A, B*). This factor evidently carries with it a considerable amount of red. Possibly the apparent intensification of the reds is nothing more than the addition of more red—that is, R and I may each represent distinct red factors. Plate C, figure *B*, shows a bright violet of phenotypic formula EEBBRRii, in which the intensifying factor I is absent. But in a similar series (EEBBRRII), Plate C, figure *A*, where it is present, much additional red is devel-

oped. The same is true for all other series containing I or i. Futhermore, when neither B nor R is present, as in Plate C, figure *G*, red pigment develops in the eye, probably owing to the factor I, bringing in the red, and the factor causing a dark eye. E acts upon I to produce the pink (red) eye.

I seems to have affected the reds (R), but not the blues nor the red associated with the blue making purple, represented by factor B. For example, the plants with the phenotypic formula eeBBrrii (Pl. C, fig. *K*) seem to be the same, the I having nothing to act upon, E or R, to cause more red to appear. The same is true of EEbbrrIi and eebbrrii (Pl. C, fig. *H*); I has no effect, and both appear as white.

The Observed and Calculated Ratios

The 543 plants that comprised the F_2 generation were divided as accurately as possible into groups, each group containing plants which were similar in appearance. This was done long before any explanation was found to account for their inheritance. Therefore, the writer was not prejudiced in the slightest degree in making his selections. This division of this population into groups was exceedingly difficult. The boundaries of the groups were not clear-cut, and very many border plants were found which were not easy to classify. This difficulty of classification may account for some of the differences that are seen between certain observed and calculated ratios. These are not so serious, however, to the man who has worked with the plants and tried to classify them. He is surprised not so much at the differences but at the nearness to theoretical ratios in most cases. For example, the writer found it very difficult to divide a certain number of bluish or purplish plants into their proper groups. The extremes of these groups could be readily recognized, but there were very many plants which might, so far as observation was concerned, go as well into one group as another. But the writer made the grouping as correctly as possible, not knowing what the interpretation would be. This accounts for the small number of plants in type B (Pl. C) and the large number in type A (Pl. C). There were many plants which might be equally well classed in either group. Other differences may be accounted for in this way.

The writer is by no means certain that all of the differences between observed and calculated ratios can be thus accounted for. There may be present linkage or repulsion, but neither has been detected as yet. The interpretation of the observations is considered merely tentative. Future crossings may modify it in many ways.

The dearth of whites may perhaps be accounted for. The plants with white flowers in this series were noticeably the weakest, and inasmuch as many plants died during the prosecution of the experiment, it is likely that a considerable proportion of these were whites.

The weakness of white-flowered plants is a common observation among gardeners. Hottes, for example, says that white varieties of the gladiolus are so weak that it is almost impossible to propagate them.

If the classes are grouped together, the individual differences tend to disappear. For instance, if all of the plants with dark-eyed flowers (E)

are put in one class and the white-eyed ones (e) in another class, the results will be as follows:

	E	e
Observed	373	170
Calculated	407. 2	135. 8

A slight discrepancy occurs because the whites fall into two classes, some supposed to have E and others e. The total number of whites are split up into classes arbitrarily for the calculation. The same was done in a few other cases.

Similarly, if the other dominants and recessives are brought together, the results are the following:

	E	e	B	b	R	r	I
Observed	373	170	404	139	442	101	399
Calculated	407. 2	135. 8	407. 2	135. 8	407. 2	135. 8	407. 2

	i	Dominants.	Recessives.
Average of all	{144	404. 5	138. 5
	{135. 8	407. 2	135. 8

SERIES II

POLLEN PARENT.—The same variety, Carnea, which was used as pollen parent in Series I and III.

SEED PARENT.—A bluish lilac variety known in commerce as Coccinea. This is a dark-eyed variety, the center of the flower being more dense than the remainder of the flower, but without any color pattern (Pl. D).

TABLE II.—*Color of the progeny of two varieties of Phlox drummondii, the Coccinea and the Carnea*

Parent or progeny.	Phenotypic formula.	Phenotype.	Field count.	Calcu- lated.	Ratio.
Seed parent: *Phlox drum- mondii*, var. Coccinea.	EBr	Bluish lilac (138–1), dark eye.			
Pollen parent: *Phlox drum- mondii*, var. Carnea.	eBr	Pale rosy pink (129–1), white eye.			
F₁ generation	EeBbRr	Carmine purple (156–2), dark eye.			
F₂ generation: Alternative factors— B {R	EEBBRR	Crimson carmine (159–3), dark eye (Pl. D, fig. *A*).	58	48. 52	27
{r	EEBBrr	Bluish lilac (183–1), dark eye (Pl. D, *B*).	18	16. 17	9
b {R	EEbbRR	Rosy white (8–1), dark eye (Pl. D, fig. *C*).	3	16. 17	9
{r	EEbbrr	White, dark (pink) eye (Pl. D, fig. *D*).	1	5. 39	3
B {R	eeBBRR	Rosy magenta (169–1), white eye (Pl. D, fig. *E*).	19	16. 17	9
{r	eeBBrr	(Perhaps some of the whites come in this class).	(*a*)	(*a*)	(*a*)
b {R	eebbRR	Bright rose (128–3), white eye (Pl. D, fig. *F*).	7	5. 39	3
{r	eebbrr	White {eeBBrr / eebbrr} (Pl. D, fig. *G*).	7	7. 19	4
Total			115		64

a See below.

All of the second-generation hybrids of this series contain more red than the corresponding hybrids of the former series. This additional red unquestionably comes from the Coccinea parent. There are not sufficient data to determine what this additional red is and its entire behavior in heredity.

The factors E, e, B, b, R, and r have a similar significance to that in Series I, although they are evidently not exactly the same. Plants in Series I and II having the same phenotypic formula are not identical, as may be seen from the illustrations. This indicates that the factors of the two series are not identical.

OBSERVED AND CALCULATED RATIOS

The calculated and observed ratios correspond very closely in this series. This is the more evident when the relatively large number of classes and the small number of individuals are taken into account. Grouping the data as before gives the following results:

	E	e	R	r	Pigmented	White
Observed	82	33	89	26	108	7
Calculated	86.3	28.7	86.3	28.7	107.8	7.2

SERIES III

POLLEN PARENT.—The same variety, Carnea, as used in Series I and II.

SEED PARENT.—A yellow variety known commercially as Large Yellow. The pigmentation is more dense at the center eye than at other parts of the flower (Pl. E).

TABLE III.—*Color of the progeny of two varieties of Phlox drummondii, the Large Yellow and the Carnea*

Parent or progeny.	Phenotypic formula.	Phenotype.	Field count.	Calcu- lated.	Ratio.
Seed parent: *Phlox drummondii*, var. Large Yellow.	ErY	Cream yellow (30–3), dark eye.			
Pollen parent: *Phlox drummondii*. var. Carnea.	eRy	Pale rosy-pink (129–1), white eye.			
F1 generation	EeRrYy	Rose Neyron red (119–2), dark eye.			
F2 generation: Alternative factors—					
E { R { Y	EERRYY	Deep lilac rose (151–2), yellow (dark) eye (Pl. E, fig. *A*).	26	39.69	27
y	EERRyy	Pale pink (135–1), dark eye (Pl. E, fig. *B*).	5	13.23	9
r { Y	EErrYY	Cream yellow (30–4), dark eye (Pl. E, fig. *C*).	9	13.23	9
y	EErryy	White, dark eye (Pl. E, figs. *D* and *E*).	10	4.41	3
e { R { Y	eeRRYY	Lilac purple (160–2), white eye (Pl. E, fig. *F*).	15	13.23	9
y	eeRRyy	Pale reddish lilac (131–1), white eye (Pl. E, fig. *G*).	6	4.41	3
r { Y	eerrYY	White (Pl E, fig. *H*)	23	5.88	4
y	eerryy	White (Pl. E, fig. *H*)			
Total			94		64

In addition to the factors already mentioned, we have a factor for yellow (Y), which acts only in the presence of the eye factor (E). Wherever Y is present and E is absent, only white occurs.

OBSERVED AND CALCULATED RATIOS

The observed and calculated ratios are again equal, with the exception of the preponderance of white, which is unaccounted for.

SUMMARY

PLANT COLORS IN PHLOX DRUMMONDII.—(1) White is due to the absence of pigment and to the reflection of light from the cells. (2) Green color is caused by the presence of a green pigment in the chlorophyll. (3) Yellow, cream, and related colors are due to a yellow pigment either associated with green in the chloroplasts or found alone in the chromoplasts; generally the latter. Yellow may sometimes come from the cell sap. (4) Red color may under certain circumstances be due to the presence of that pigment in the chromoplasts, but is ordinarily a cell-sap color. (5) Most of the remaining colors, purple, blue, generally red, pink, etc., are due to pigments in the cell sap. (6) Many of the colors and shades found in flowers are the result of both plastid colors and cell-sap colors acting together in various proportions. (7) Certain of the denser plastids or cell-sap colors may cover up the more delicate colors so that they can not be seen. (8) Finally, the color in the cell sap may be due to the relative presence of a non-nitrogenous chemical substance, anthocyanin. This is blue in alkaline and red in an acid-reacting cell sap and, under certain conditions, also dark red, violet, dark blue, and even blackish blue. Anthocyanin can be obtained from the supersaturated cell sap of a number of deeply colored parts of plants in a crystalline or amorphous form. Blood-colored leaves, such as those of the copper beech, owe their characteristic appearance to the united presence of green chlorophyll and anthocyanin. The different colors of flowers are due to the varying color of the cell sap, to the different distribution of the cells containing the colored cell sap, and also to the combinations of dissolved coloring matter with the yellow, orange, and red chromoplasts and the green chloroplasts. There is occasionally found in the cell sap a yellow coloring matter known as "xanthein," nearly related to xanthophyll, but soluble in water.

COLOR INHERITANCE IN PHLOX DRUMMONDII.—The following unit characters were found in the four varieties of *Phlox drummondii* that were used in these experiments: (1) A dark eye factor producing a dense coloration at the center of the flower. This was dominant over its absence, the white eye, which was exhibited in more or less of a definite pattern. (2) A blue factor. (3) A red factor. (4) An intensifying factor which determines the degree of pigmentation of the reds. (5) A yellow factor which acts only in the presence of the eye factor.

The reds and blues are cell-sap colors, and the yellow is due to the presence of yellow chromoplasts.

Studies of this nature will eventually lead to a time when color and color inheritance are sufficiently understood and controlled to be of great commercial value to the florist or grower of ornamental plants.

PLATE C

PLATE *D*

Phlox drummondii:

Fig. ♀ —Seed parent, Coccinea variety.
Fig. ♂ —Pollen parent, Carnea variety.
Fig. F_1—First-generation hybrid between Coccinea and Carnea.
Fig. A to G—Second-generation hybrids between Coccinea and Carnea.

ASPARAGUS-BEETLE EGG PARASITE

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INTRODUCTION

On May 23, 1909, a minute chalcidid parasite was reported from Concord, Mass., by Messrs. C. W. Prescott and J. B. Norton, of the Bureau of Plant Industry, where, according to information received from Mr. Prescott, the insect was observed devouring the contents of the eggs of the asparagus beetle (*Crioceris asparagi* L.). Later, on June 2, Dr. H. T. Fernald found the same parasite at Amherst, Mass. The species was referred to the Bureau of Entomology and was determined by Mr. J. C. Crawford, of the United States National Museum, as being new to science, and was accordingly described as *Tetrastichus asparagi* Cwfd. (1).[1] In July of that year and later, in August, Dr. Fernald published short articles on this species. Since the asparagus beetles have never been carefully studied throughout their life history, the fact that the parasite had been recorded was overlooked. In an earlier article, however, published in 1869, Riley and Walsh (6) referred to a notice of the occurrence of a parasitic fly as follows:

But in the year 1863, as we learn from Isaac Hicks, of Long Island, a deliverer appeared in the form of a small shining black parasitic fly, probably belonging either to the Chalcis or to the Proctotrupes family. Whether this fly lays its eggs in the eggs of the asparagus beetle or in the larva of that insect does not seem to be at present clearly ascertained; but if the accounts that we have received of it be correct it must do either one or the other. In the former case the larva that hatches out from the parasitic egg will consume the egg of the asparagus beetle and entirely prevent it from hatching; in the latter case it will destroy the larva before it has time to pass into the perfect state. The result in either event will be equally destructive to the bug and beneficial to the gardener.

Later, in 1882, Lintner (4) made notes on the same species, referring to the publication in the American Entomologist just quoted. Again in 1893 (5) he called attention to a parasite, stating that it was undescribed and that it might have disappeared before it could receive scientific attention, because nothing seemed to be known of it at that time.

From the descriptions given it seems almost certain that the parasite mentioned by Riley and Walsh and *Tetrastichus asparagi* Cwfd. are the same. If so, it is hard to explain why this insect, which was reported in considerable numbers in 1863, should have escaped further observation until 1909.

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[1] Reference is made by number to "Literature cited," p. 312.

So far as known by the writer, the life history of this parasite had not been studied, and as it appeared in considerable numbers during the season of 1912 near Riverhead, N. Y., where the writer was stationed, a study of its life history and habits was undertaken. During the fall of 1912 Mr. H. M. Russell, of the Bureau of Entomology, also stationed at Riverhead, and the writer published a short article (7) on this parasite, based principally on observations made during that season.

DESCRIPTION OF THE PARASITE

The adult (Pl. XLIX, fig. 1) of *T. asparagi* was described by Mr. Crawford as follows:

THE ADULT

Female.—Length 2 mm. Belongs to the group of *T. hylotomae* Ashm.; dark blue green; face finely reticulate and with scattered punctures; antennæ with one ring joint; joints 1–3 of flagellum almost equal, the first slightly longer and about as long as the pedicel; third flagellar joint hardly longer than wide and about as long as the first joint of club; mesothorax finely longitudinally rugulose, the median furrow failing anteriorly; middle lobe of mesonotum with a single indistinct row of punctures on each side; metathorax roughened, median and lateral carinæ strong; metathorax at median carina much longer than postscutellum; coxæ, trochanters, and femora, except apices, green, the rest of the legs reddish testaceous.

Amherst, Mass., reared from eggs of *Crioceris asparagi* by Dr. H. T. Fernald.

Type.—Cat. No. 12676, U. S. Nat. Mus.

This species is very closely related to *T. hylotomae*, but has shorter antennæ. In the female of *T. hylotomae* the third joint of the flagellum is twice as long as wide, and distinctly longer than the first joint of the club, the first joint of the flagellum is one and one-half times as long as the pedicel; the median furrow of mesonotum is distinct to the anterior margin.

The following descriptions of the egg, larva, and pupa of *Tetrastichus asparagi* are by the author.

THE EGG

The egg (Pl. XLIX, fig. 2) is reniform in shape, with one end more slender than the other, about 0.24 mm. long and 0.08 mm. wide, semitransparent, and is of a milky color, with a granular appearance within. While the eggs may be laid singly, in a number of cases they were found side by side in pairs (Pl. XLIX, fig. 3).

THE LARVA

The mature larva (Pl. XLIX, fig. 4) is from 2 to 2.5 mm. long and about 1 mm. wide. It is white, with the alimentary canal appearing greenish; ovate; widest near the head, which is contracted and bent under the body. The surface is smooth and devoid of hairs. There are no legs, and the larva seems incapable of motion, except to move the end of the abdomen when disturbed.

THE PUPA

The pupa (Pl. XLIX, fig. 5) is from 2 to 2.5 mm. long and from 1 to 1.2 mm. wide. It is yellowish white; convex dorsally, with the head somewhat bent under and inconspicuous wing pads folded along the side (Pl. XLIX, fig. 6). The antennæ and legs are folded under ventrally. The head, thorax, and abdomen are distinctly differentiated from one another; the abdomen tapers posteriorly.

DISTRIBUTION OF THE PARASITE

This insect has been recorded from Amherst and Concord, Mass., by Dr. H. T. Fernald; by Mr. D. E. Fink, of the Bureau of Entomology, from Ithaca, N. Y.; and the writer has observed it in considerable numbers on Long Island. During June and July, 1911, specimens of this insect were liberated at Jessup, Riverdale, and Rives, Md., but at the present time it has not been retaken in these places. In all probability, however, it is present in many localities in the northeastern part of the United States, other than those mentioned.

OCCURRENCE ON LONG ISLAND

On June 10, 1912, the author first observed this parasite at Aquebogue, Long Island, while examining an asparagus field. Large numbers of this insect, together with the asparagus beetle (*Crioceris asparagi*) and its eggs, were found on check rows of asparagus which had been left in the field to attract the beetles from the main crop. At times as many as six or seven parasites were to be seen on a single stalk. These check rows had not been set aside as a trap for the beetles until a short time before, and as a consequence the asparagus was not over a foot high and had not branched out. As a result, the beetle eggs were confined to a limited area, and it was fairly easy to follow the actions of the parasites.

FEEDING OF PARASITES

This insect is an energetic feeder on its host's eggs and is evidently as useful in checking the host in this way as by its parasitic development, if not more so.

When a careful examination of the beetle eggs on stalks of asparagus was made, many were found that had collapsed and withered, and it was quite evident that they would never hatch. On some asparagus stalks the only viable eggs appeared to be those recently deposited. The cause of this collapsed condition of the eggs was soon apparent. A female adult parasite under observation approached an egg, and, after carefully examining it with her antennæ, climbed upon it, inserted her ovipositor, and worked it up and down with a pumping motion. This motion was continued for varying lengths of time, from a few seconds to three or four minutes, after which she withdrew the ovipositor, backed down from the egg, and, applying her mouthparts to the puncture, sucked up the egg contents.

She usually fed from the egg until the shell collapsed. At times it was necessary for her to manipulate the ovipositor in the egg four or five times before the contents were sufficiently loosened to permit their extraction. This feeding of the parasite was so extensive that of 2,097 eggs counted on 28 stalks of asparagus, 1,495, or 71.29 per cent, had been destroyed.

LABORATORY EXPERIMENTS

Several of the adult parasites, captured and confined with the eggs of the asparagus beetle in the laboratory, were noted shortly afterward, ovipositing and feeding on the beetle eggs. A few days later all eggs of *Crioceris asparagi* which had not been eaten by the parasites had hatched. At the time this could not be accounted for, since this insect had previously been considered an egg parasite and many of the eggs which hatched were known to have been subjects of oviposition. None of the young beetle larvæ that hatched from these eggs were carried through to maturity. The cause of their death appeared to be a lack of proper food.

On the first day that the parasites were observed in the field, Mr. Russell collected nearly mature larvæ of the asparagus beetle from volunteer asparagus plants in a field which had been planted to asparagus at some prior date. The larvæ were taken to the laboratory and placed in rearing cages that they might form their cells. A few days later, while the cocoons were being examined, six small whitish larvæ were found in one cocoon. Some of these larvæ at a later date pupated, but died before the adult stage was reached, so there was no certainty that these were the larvæ of *T. asparagi*.

About July 10 the writer collected asparagus-beetle larvæ from a field in which parasites had been previously noted in abundance and, bringing them into the laboratory, supplied them with food and confined them in vials without earth.

Upon examining the vials on July 24 it was seen that five beetles had emerged and in one vial there were three small pupæ. In another vial was a small whitish larva similar to those which Mr. Russell had previously taken from a beetle cell.

The three pupæ were placed in a separate vial, and on July 30 and 31 they emerged as adults, which were later identified by Mr. Crawford as *T. asparagi*.

On June 20 the writer dissected the egg of *T. asparagi* from the asparagus-beetle egg, and the peculiar life history of this parasite was at length established.

For nearly two weeks after the parasites were first observed in the field they were to be found in considerable numbers, after which they suddenly disappeared; by June 24 none could be found. During the latter part of July they again made their appearance in the field but were much harder to locate, since the entire asparagus field by this time had been allowed to grow, and, in consequence, the parasites were scattered over a much larger area than before.

On August 5 the first parasites of a second generation were captured in the field and brought into the laboratory. They were confined in large vials and each day were given a fresh supply of beetle eggs, which were treated as noted before. On hatching, the beetle larvæ were removed to

another vial and supplied daily with fresh food. At maturity they went into the soil in the bottom of the vial, and in due time either adult beetles or the parasites issued from the soil.

METHODS USED FOR REARING THE PARASITES IN CONFINEMENT

The adults of the parasite *T. asparagi* were captured in the field and confined in the laboratory in vials, measuring 100 mm. in length and 28 mm. in diameter. The ends of the vials were covered with cheesecloth, as better results were obtained when this was used than when the vials were stopped with cotton plugs. Each day a supply of fresh asparagus-beetle eggs was collected in the field, brought into the laboratory, and a certain number placed in a vial with each parasite. The eggs remained with the parasites for 24 hours, when they were removed, the number parasitized and the number eaten by each parasite being recorded and the twigs bearing the eggs placed in moist sand, so that the eggs might hatch.

As soon as the young beetle larvæ hatched, they were confined in vials about one-third full of moist earth, and supplied with fresh food each day. As soon as the beetle larvæ were full grown, they were allowed to go into the soil in the vial and pupate. In several cases the pupal cells were formed near the glass, and it was possible to observe the naked parasitic larvæ in the cell after they had completely consumed their host.

LENGTH OF LIFE OF ADULTS IN CONFINEMENT

Tables I, II, and III show the length of life of adults collected in the field and those reared in the laboratory, and the number of eggs parasitized and eaten by each parasite.

TABLE I.—*Length of life of the parasite Tetrastichus asparagi, reared at the Riverhead, N. Y., laboratory in 1912*

Date of emergence.	Date of death.	Number of eggs parasitized.	Number of eggs eaten.	Length of life.[a]	Average number of eggs parasitized daily.	Average number of eggs eaten daily.
				Days.		
Sept. 3	Sept. 9	18	26	[b]6	3	4. 33
3	9	16	30	6	2. 66	5
3	9	14	32	6	2. 33	5. 33
3	10	11	25	7	1. 57	3. 57
3	8	15	20	[c]5	3	4
3	10	11	22	7	1. 57	3. 14
12	17	0	0	5	....	....
12	17	0	0	5	....	....
12	18	10	10	6	1. 66	1. 66
12	22	19	27	10	1. 99	2. 7
12	18	0	1	6	....	....
12	Oct. 7	40	61	[d]25	1. 81	2. 77

[a] The average length of life was 7.83 days: the maximum, 25 days.
[b] All died on Sept. 9, when they were left from morning until afternoon without any eggs in the vial.
[c] This parasite was accidentally killed.
[d] During the last 3 days no asparagus-beetle eggs could be obtained.

TABLE II.—*Length of life of adult Tetrastichus asparagi at the Riverhead, N. Y., laboratory in 1912*

Date of capture.	Date of death.	Number of eggs para-sitized.	Number of eggs eaten.	Length of life.[a]	Average number of eggs parasi-tized daily.	Average number of eggs eaten daily.
				Days.		
Aug. 5	Aug. 11	0	7	[b]6	0	3. 5
5	12	9	11	[c]7	3	3. 66
7	11	13	22	4	3. 25	5. 5
7	14	22	44	7	3. 14	6. 28
8	19	41	50	11	3. 72	4. 54
8	19	17	51	11	1. 54	[d]4. 63
12	14	0	6	2	0	3
12	17	16	28	5	3. 2	5. 6
12	26	7	37	14	. 5	[d]2. 64
17	21	0	9	4	0	2. 25

[a] The average length of life was 7.1 days; the maximum, 14 days.
[b] Records for the last 2 days only.
[c] Records for the last 3 days only.
[d] Several eggs hatched in the parasite vials.

TABLE III.—*Life cycle of Tetrastichus asparagi at the Riverhead, N. Y., laboratory in 1912*

Date of ovi-position.	Date of emergence.	Number emerged.	Length of stages.	Remarks.
			Days.	
Aug. 6	Aug. 30	6	24	Was removed from the soil.
6	30	6	24	
8	30	[a] 1	....	
8	Sept. 2	5	25	
8	3	6	26	
9	2	7	24	
9	5	5	27	
11	5	4	25	
11	6	6	26	
6	6	2	31	
6	7	2	....	Dead adults taken from top of the soil.
9	7	5	29	
6	11	[b] 7	....	The seven adults were dead in the soil.
15	12	6	28	
15	15	6	31	
15	16	[c] 2	....	Were taken from dirt near top of vial.
17	16	7	30	
6	21	....	....	Four larvæ and nine dead adults taken from soil in vial.
8	25	....	....	Four larvæ dug out of soil in vial.
Sept. 5	Oct. 11	7	36	Second generation.

[a] Pupa.   [b] Adults.   [c] Dead.

SEXES

During the time that this parasite has been under observation only females have been found, both among the adults collected in the field and among those reared in the laboratory, and reproduction has been parthenogenetic, so far as has been observed. Of two generations that have been reared in the laboratory, no males have appeared.

Parasites which were separated as soon as they emerged, and confined with asparagus-beetle eggs, immediately commenced feeding and ovipositing and another generation was reared from the parasitized eggs. In one case where six parasites emerged in one vial and were immediately separated, each being given beetle eggs, five of the six were observed to feed on an egg within 15 minutes after they were placed with the eggs, while the other one was observed to oviposit first. From this it would seem that, as a rule, the parasite after emerging feeds on a few eggs before beginning to oviposit. These five adults were observed to oviposit later in the day.

NUMBER OF GENERATIONS

Apparently this insect produces three generations a year on Long Island, for it was very abundant early in June, when it disappeared, to be found again in July, after which time two generations were reared in the laboratory. However, indications are that the third generation, in the fall, is only a partial one.

In one case three beetle eggs were found to be parasitized on August 9, and on August 11 the beetle larvæ hatched and were given food. On August 17 they went into the ground to pupate, and on September 7 five adult parasites emerged. On January 3, 1913, when the soil in the vial was taken out and examined, it was found that one of the two cells which were still in the ground held five dead adult parasites. In the other cell there were five parasitic pupæ. These pupæ, being confined in a warm room, immediately began to change and on January 8 emerged as adults. In another case, from beetle larvæ hatched on August 11 from eggs that had been confined with one parasite, 10 parasites emerged on September 5 and 6. As no more issued from this vial, the soil in it was taken out and examined February 3, 1913. In one cell were found five parasitic larvæ. These larvæ pupated February 7 and on February 17 were emerging as adults. From these facts it would appear that the last generation was but a partial one. The fact that in the laboratory experiments representatives of the third generation emerged in one vial only, whereas on examining the soil in some of the vials during January a number of parasitic larvæ were found, would indicate that the third generation might be the exception instead of the rule.

HIBERNATION

During the latter part of January and the first of February the soil in several vials was examined in order that the stage in which this insect passed the winter might be ascertained. Seven cells containing parasites were found, in six of which they were in the larval stage, while in the seventh they had passed to the pupal stage. This would indicate that the insect hibernates as a full-grown larva in the cell of its host in the ground.

NUMBER OF PARASITES EMERGING FROM SINGLE HOST LARVA

In dissecting eggs of the host, from 1 to 5 eggs of the parasite were found, and in the rearing experiments undertaken in the laboratory from 1 to 10 larvæ of the parasite have been found in a single beetle cell. However, the usual number of parasites that issued from one host larva was from 5 to 7. In two cases only were more than 7 parasites found in a single host and in 1 of these 10 and in the other 9 were found. There was one case where only 1 parasite was found in the host, but as mites had destroyed several cells in this vial and were also in this cell, it seems strongly probable that they had destroyed some of the parasites in this particular cell.

ONLY HOST

The asparagus-beetle egg parasite has been observed attacking only the eggs of the common asparagus beetle (*Crioceris asparagi*). In the laboratory it has been confined with the eggs and young larvæ of the potato beetle, and with the eggs of the elm leaf beetle, but it paid no attention to them.

PUPATION AND THE PUPAL PERIOD

The pupa when first formed is yellowish white throughout. Shortly the eyes become reddish and the mandibles darken. In from two to three days the eyes are bright red and the ocelli are also visible and are of the same color. Next, the head and thorax begin to turn black and this continues on through the abdomen, until just before emergence the whole pupa appears black.

Parasitic larvæ which were seen in a cell on August 20 emerged as adults on August 30. Another brood first seen on August 26 on September 7 emerged as adults.

In a vial in which parasite larvæ were seen on August 20, adult parasites emerged August 30.

Parasitic larvæ which were taken from the soil on January 25, 1913, and kept in a warm room pupated on January 30 and the adults emerged on February 8.

Another lot of larvæ taken from the soil on February 3 pupated on February 7 and emerged as adults on February 17. According to these data, the pupal period lasts from 7 to 11 days.

OVIPOSITION

The process of oviposition is in some respects different from that of feeding. The parasite crawls slowly over the plant with the antennæ held down in front of the face and kept in constant vibration. When a beetle egg is encountered it is carefully examined with the antennæ and, if satisfactory, the parasite crawls upon it and inserts the ovipositor. The ovipositor remains in the egg for a few minutes, without the pul-

sations of the abdomen noticed when the parasite feeds on an egg. It is then withdrawn, and the parasite leaves the egg. In one or two instances it appeared that the parasite after ovipositing in an egg returned to it and repeated the act of oviposition.

In another case a parasite observed in the act of oviposition was approached by a second individual which, climbing up on the opposite side of the egg, began to work the ovipositer up and down in the egg in preparation for feeding. Each was aware of the other's presence, but paid no observable attention to the other.

Table IV gives the time required for oviposition and feeding for a few individuals.

TABLE IV.—*Length of oviposition and of feeding of Tetrastichus asparagi at the River-head, N. Y., laboratory in 1912*

Length of oviposition.						Length of feeding.		
Parasite No.	Minutes.	Seconds.	Parasite No.	Minutes.	Seconds.	Parasite No.	Minutes.	Seconds.
I	....	33	12	I	a42	I	23	30
2	2	5	13	2	15	2	8	30
3	3	25	14	3	32	3	11	35
4	2	15	15	2	25	4	9	27
5	I	....	16	3	30	5	9	....
6	2	30	17	4	43	6	6	....
7	....	40	18	....	25			
8	....	a55	19	4	....			
9	2	a25	20	4	12			
10	I	10	21	3	10			
11	....	a36	22	3	....			

a Same eggs.

EVIDENCE OF PARASITISM

By means of a hand lens the beetle eggs which had been parasitized were readily distinguishable. Where the ovipositor punctured the egg, a small circular area appeared which projected slightly from the rest of the eggshell and which had a shiny appearance, caused by the small amount of the contents of the egg which had oozed from the puncture.

Beetle larvæ hatching from parasitized eggs appeared normal and continued to feed and grow until maturity. When matured, they went into the ground and prepared their cells for pupation, but here their activities stopped, and in a few days the cell was occupied by the parasitic larvæ, all that remained of the beetle larva being the empty skin.

IMPORTANCE OF THIS PARASITE

The asparagus-beetle egg parasite is of considerable importance, as it not only attacks the host during its parasitic development but is also beneficial in destroying its host's eggs through feeding; in fact, it ap-

pears to be of greater value as an egg destroyer than as a parasite developing within the host.

Mr. C. W. Prescott, of Concord, Mass., recently wrote that on May 23, 1909, he had noticed the parasite in the field feeding on the host eggs, and that on the day of writing he had attempted to find "slugs" or larvæ, but could find neither slug nor egg except those absolutely dry, in a field of 5 acres.

During the season of 1912, the field of asparagus at Aquebogue, N. Y., where this insect was found, received no treatment for the beetles, yet these were so scarce that no damage resulted. Previous to this, according to the owner, the field had been sprayed each year to prevent serious injury.

Without doubt this parasite was to a large degree responsible for the scarcity of the asparagus beetles. Certain other factors may have assisted, but there is little doubt that the parasites were the most important factors in preventing damage.

SUMMARY

Previous to the time that *Tetrastichus asparagi* was believed to be an egg parasite, its life history had never been worked out. As the parasite had been observed ovipositing in the host egg, it was supposed that it developed in the egg. During the investigation of the life history it was discovered that this insect presented one of those peculiar instances where oviposition in the host's eggs and retarded development of the parasite permitted the host to develop. In this case the following takes place:

The parasite deposits her eggs in the egg of the asparagus beetle; the beetle egg hatches; its larva feeds to maturity and entering the soil forms a pupal cell, but does not pupate. The parasites have by this time totally consumed the larva and emerge from it into the cell the larva has constructed, where they pupate and later emerge as adults.

Since the parasitic larva passes the winter in the soil in the pupal cell of its host, it would appear that the parasite might easily be transported from one place to another in the soil which might surround a shipment of asparagus roots.

LITERATURE CITED

(1) CRAWFORD, J. C.
>   1909. Two new species of the genus Tetrastichus (Hymenoptera, Eulophidæ). *In* Proc. Ent. Soc. Wash., v. 11, no. 3, p. 150.
>
>> Technical description of adult female of *Tetrastichus asparagi*, n. sp., from Amherst, Mass.

(2) FERNALD, H. T.
>   1909. A parasite of the asparagus beetle. Mass. Agr. Exp. Sta. Circ. 23, 2 p.
>
>> Brief account of discovery and oviposition and observations on some of the habits of the adult.

(3) ——
>   1909. A parasite on the asparagus beetle. *In* Jour. Econ. Ent., v. 2, no. 4, p. 278–279.
>
>> Substantially the same as preceding article.

(4) LINTNER, J. A.

1882. Crioceris asparagi (Linn.). The asparagus beetle. *In* 1st Ann. Rpt. Injur. and Other Insects N. Y., p. 239–246, figs. 70–72.

Notes the parasite recorded by Riley and Walsh.

(5) ————

1893. The asparagus beetle. *In* 8th Rpt. Injur. and Other Insects N. Y., 1891, p. 250–253.

Similar to the preceding article.

(6) RILEY, C. V., and WALSH, B. D.

1869. The asparagus beetle (Crioceris asparagi, Linn.). *In* Amer. Ent., v. 1, no. 5, p. 114–115.

Mention is made of a hymenopterous parasite, referred to Chalcis or Proctotrupes, presumed to be this species, appearing on Long Island.

(7) RUSSELL, H. M., and JOHNSTON, F. A.

1912. The life history of Tetrastichus asparagi Crawf. *In* Jour. Econ. Ent., v. 5, no. 6, p. 429–432.

Short article on life history with description of different stages, oviposition, and feeding habits of adults.
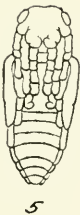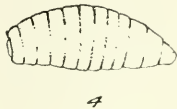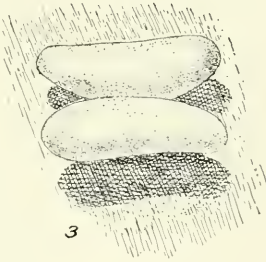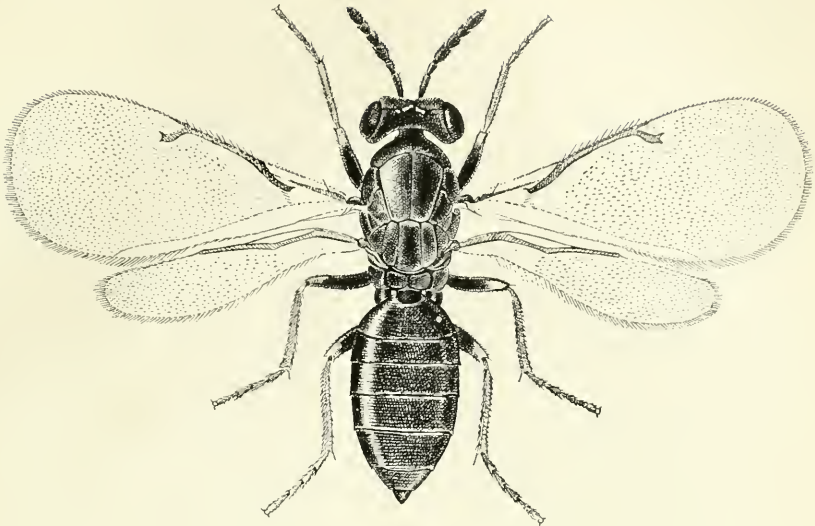
INHERITANCE OF CERTAIN CHARACTERS OF GRAPES

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INTRODUCTION

The breeding of grapes (*Vitis* spp.) was begun in the Horticultural Department of the New York Agricultural Experiment Station about 25 years ago and has been continued throughout this time as a horticultural problem. Nearly 10,000 seedlings have been grown, and of these about 6,000 have fruited. This work was begun about 1885 by Prof. E. S. Goff, who at first grew seedlings and plants from seeds open to cross-pollination. Later he crossed a number of varieties. In 1891 Prof. S. A. Beach became the Station horticulturist, and besides seeking to obtain new varieties he made studies of self-sterile varieties, studied the correlation between the size of seeds and vigor of plants, and did considerable hybridizing. In 1905 the senior author took charge of the work in horticulture at the Station. Mendel's work had just been discovered, and plant breeding was undergoing a stimulus from it. The work with grapes was therefore replanned and extensively added to with a view to studying problems of inheritance. This work has been continued and increased from year to year. Several assistants and associates have spent much of their time working with grapes at this Station. Mr. N. O. Booth worked with grapes from 1901 to 1908; Mr. M. J. Dorsey from 1907 to 1910; Mr. Richard Wellington from 1906 to 1913; Mr. R. D. Anthony, the junior author, began work at this Station in the summer of 1913, and has devoted most of his time to the grape work since then. Upon him has fallen the task of presenting the data in this paper. It is the purpose of this paper to discuss certain results of this work.

AIMS, METHODS, AND MATERIALS

During this quarter of a century, experience and a better understanding of the principles of breeding have modified many of the methods and changed considerably the nature of the data which are now taken.

The ultimate aim in this work is, of course, the production of improved horticultural varieties. Through the early days, when breeding laws and methods were less understood than now, there was a tendency to make this the immediate as well as the ultimate aim. The fact that the first 20 years of grape breeding produced but one variety worthy a name served to confirm the conviction that this goal would be reached quicker by for-

getting it for the time being and bending every effort to the discovery of how grape characters are transmitted.

The work is now proceeding mainly along two lines: (1) The determination of the breeding potentialities of a considerable number of varieties of grapes, especially with the view of finding unit characters; and (2) a review of all the New York Experiment Station breeding data on this fruit, to study and interpret breeding phenomena, accompanying this review with the making of the crosses necessary to throw further light upon doubtful points.

The results secured in testing the breeding possibilities of grape varieties, which will be discussed later, have made it seem desirable to extend this study to all the varieties which show any promise. For this reason nearly 200 different kinds have been used as pollen parents and nearly 100 as maternal parents in this work.

Frequently during the early days of the work seedlings which seemed to lack vigor in the nursery were discarded, instead of being planted in the test vineyards. Though this undoubtedly removed many unpromising seedlings, it seriously decreased the number which fruited, and made the interpretation of results difficult and uncertain. At best the number of seedlings that lived of each cross was smaller than could be desired, and, when this number was still further decreased by selection in the nursery or by untoward circumstances, much of the value of the work from a breeding standpoint was lost.

Another change of method which bids fair to be exceedingly important has been the use of varieties of *Vitis vinifera* in breeding. Every indication points to the desirability of the addition of some of the blood of the European grape to our native sorts. Although we are working primarily to determine breeding laws, there is usually a wide choice of varieties which answer our purpose, and with the growing of nearly 100 varieties of this species on the New York Station grounds we have been able to use several as parents. There are now several hundred hybrids containing *V. vinifera* blood growing on the Station grounds, and these will be increased by many hundreds during the following years.

The methods used in the actual work of crossing are similar to those of most breeders. The female blossoms are emasculated before the calyx cap splits off and are then bagged; the male blossoms are also bagged before the calyx splits. When the pollen is ripe, the bagged male cluster is usually cut from the vine and all or part of it brushed over the emasculated female. Usually some of the male cluster is inclosed in the bag, which is again put over the female after pollination. In a few cases, where the periods of blossoming of two varieties are too widely separated, it has been necessary to save pollen in clean glass jars. It is customary to dip the forceps used in pollination into alcohol with each new variety.

Certain results secured in the summer of 1914 seem to indicate that this method is perhaps open to criticism. While emasculating clusters of the Janesville variety it was found that, although the cap had not split, the pollen in the anthers seemed to be mature, and, as the anthers were ruptured during the emasculation, there was a possibility of self-fertilization taking place. Several clusters were emasculated and bagged without being pollinated. These set nearly the full quota of berries with seeds that have every appearance of being viable. With two other varieties, clusters emasculated and not pollinated matured a few plump seeds, though the clusters were much below normal. A somewhat similar instance is reported by Beach (1),[1] the variety being the Mills. This point deserves careful study, for if it is found that serious danger of self-pollination exists before the calyx cap splits it will be necessary to change the method—at least to the extent of emasculating the clusters several days before the cap is ready to come off.

All data regarding size and shape have been recorded in comparative terms, instead of the actual measurements being taken. With a limited number of observers and thousands of seedlings of the various fruits to be studied each year, it was a physical impossibility to take measurements and would not have increased the value of the records to any extent from a horticultural point of view, though it would, of course, have furnished interesting material for a statistical study. The value of data reported in comparative terms depends upon the accuracy of the recorder. The work with the grape has always been done by members of the scientific staff, and the observations have usually been checked during several seasons.

GENERAL RESULTS OF THE STUDY OF VARIETIES OF GRAPES

One of the surprises in the study of varieties of grapes was the failure of many of our commercial sorts to transmit desirable qualities to their progeny. Seedlings of Concord, Niagara, Worden, Delaware, and Catawba grapes have so far proved only disappointments. The best results have been secured from such little-grown varieties as the Ross, Collier, Mills, Jefferson, Diamond, and Winchell. This has made it seem desirable to test all varieties that show any promise. The first step, then, was to secure as many varieties as possible which were of any value and which could be grown in northeastern United States. More than 400 such varieties have fruited in the Station's vineyards and have been described. About 200 of these have been used to a greater or less extent in the breeding work.

As an aid in studying the breeding possibilities of grape varieties the Station has grown nearly 3,000 selfed, or pure, seedlings, using as parents most of those varieties which have entered into the crosses that have been

[1] Reference is made by number to "Literature cited" p. 329-330.

made. These seedlings have thrown much light upon the inheritance of various factors, but they have been so uniformly lacking in vigor as to lead to the belief that only through crossbreeding can we hope to produce improved varieties.

INHERITANCE OF CHARACTERS

The grape characters discussed in the following pages are those for which sufficient data are available to make such a discussion of value.

SELF-STERILITY

On the basis of flower type, grapes may be divided into three classes: (1) True hermaphrodites; (2) hermaphrodites functioning as females, owing to completely or partially abortive pollen; and (3) pure males with the pistil absent or rudimentary. Among these classes there are two types of stamens: Those with upright filaments and those in which the filaments bend backward and downward soon after the calyx cap falls off. According to Dorsey (5), this is due to the cells of the outer surface of the reflexed stamens being smaller and having thinner walls.

So far as observed at the New York Station, all pure males have upright stamens. Among the two classes of females Beach (1) found that only those varieties with upright stamens were capable of producing marketable clusters of fruit when self-fertilized. At the same time he reported nine varieties with upright stamens to be self-sterile. Since Beach published his report further work at this Station with three of these nine varieties has proved them self-fertile, and it is probably safe to say that all varieties with upright stamens are self-fertile, though in varying degrees. Reflexed stamens are always correlated with complete or nearly complete self-sterility. Reimer and Detjen (6) found this last conclusion to hold also with *Vitis rotundifolia*, a species not studied at this Station.

The cause of self-sterility in varieties with reflexed stamens seems to be a lack of viability in all or a large part of the pollen of such varieties. Booth (2) found that such pollen was quite irregular in form and would not germinate in sugar solutions. Reimer and Detjen (6) state "the pollen of all the present cultivated [female] varieties [of *V. rotundifolia*] is worthless." Recently Dorsey (4) has ascribed the cause of this self-sterility to a degeneration in the generative nucleus. While this impotency may be absolute in many of the varieties, in some at least it is only relative. Frequently viable pollen will be found mixed with the usual misshapen, abortive pollen of the self-sterile varieties, and nearly a hundred pure seedlings of the varieties which Beach (1) reported as totally self-sterile have been grown in the Geneva Station vineyard. The degree of sterility seems to depend to some extent upon the condition of the vine due to environmental factors.

From a practical standpoint it is undesirable to grow self-sterile varieties. They will not succeed in the large blocks of the commercial plantation, nor are they always properly fertilized in the small home vineyard. Can we, then, in our grape breeding eliminate self-sterility? Letting U stand for upright stamens and R for reflex, the following table gives the results of our crosses:

U×U[1] =180U+ 47R   R×R = 16U+16R   R×U =207U+206R
U selfed=691U+152R   R selfed= 94U+73R   Ratio ....1U : 1R

Total...871U+199R   Total...110U+89R   U×R=?
Ratio .....4.3U : 1R   Ratio .....1.2U : 1R

Of the varieties whose pure seedlings have entered into the ratio of 4.3 U to 1 R, only two, involving 18 seedlings, have given simply upright stamens; consequently it may safely be said that no variety has proved pure for upright stamens. In the remaining crosses of this class the ratios have ranged from 1 U to 2 R up to 10 U to 1 R, with the greatest frequency at 2 U to 1 R. The results with the crossed seedlings are practically the same. Over a thousand seedlings from crosses of one type would be expected to give some rather definite results; yet these results are anything but definite, and apparently no conclusions can be drawn from them except that the varieties are not homozygous for uprightness of stamens.

The ratio of practically 1 to 1 in crosses of varieties with reflexed stamens is perhaps best accounted for by the supposition that the gametic composition of pollen and ovules is not alike. The ratio of 1 to 1 in crosses of reflexed stamens by upright may be covered by the same assumption. It should be noted that the pollen of the upright varieties produces the same ratio as that of the reflexed varieties when both are used on ovules of the reflexed kinds.

Upright varieties have been crossed many times with reflexed sorts and several hundred seedlings should have resulted from these, yet only one plant has survived the vicissitudes of the seed bed and nursery to be planted in the test vineyards. In the last five years 50 crosses have yielded 600 seeds; yet from these there are now in the nurseries but 25 living seedlings. Many of the pollen parents used in these crosses were the same as those used in the cross R × R. In the two crosses R × R and R × U, the pollen from upright and reflexed varieties produced the same results; but comparing this last case, U × R, with the first one, U × U, we see that the pollen of the upright and reflexed varieties has produced quite different results when used on upright sorts. Why this should be is not apparent.

At present there does not seem to be any way of eliminating reflexed stamens, but we can at least decrease the proportion by using for breeding only varieties with upright stamens.

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[1] The pollen parent is always placed last.

INHERITANCE OF SEX

Among more than 6,000 seedlings which have flowered in the New York Experiment Station vineyards, less than 100 pure male vines have been found. Of these there are complete breeding records for 62 vines, 51 of which came from crosses in which the pollen parent was a pure male, leaving 11 males recorded as produced by pollen from hermaphrodite plants. Of these, 5 were pure seedlings from one parent, the other 6 from 5 crosses. These 6 were probably hermaphrodites erroneously recorded as males, an error very easy to make when the pistil has a short style and one which has been made several times and corrected by subsequent observations. The parent yielding the 5 males was discarded shortly after being used in breeding, and our records are meager. It was probably an intermediate recorded as a hermaphrodite. Such an intermediate, having both male and hermaphrodite blossoms, is under observation in one of the Station vineyards, and its pollen seems to behave as the pollen of a pure male; in other words, it is reasonable to assume that, excluding these intermediate forms, pollen from hermaphrodite plants will not produce pure males.

The results obtained from pure males as pollen parents are:

Hermaphrodite female $\times$ pure male =
56 hermaphrodites + 51 pure males

Following the assumptions usual to such cases, the hermaphrodite would be considered a sex heterozygote and the male a sex homozygote. Yet selfed hermaphrodites yield only hermaphrodites. These results are similar to those obtained at this Station from selfed hermaphrodite strawberries, but differ from Shull's results (7) with species of Lychnis, where the hermaphrodite gave both females and hermaphrodites. This condition might be covered by the assumption that the hermaphrodite is a female in which the addition of a single dose of maleness has caused the production of male organs, the ovules keeping the composition ♀ ♀ and the pollen becoming ♂ ♀ :

Hermaphrodite $\times$ hermaphrodite = ♀ ♀ $\times$ ♂ ♀ = 2 ♂ ♀ + 2 ♀ ♀

Since we have no pure females, we must assume that some condition prevents the formation of individuals with the composition ♀ ♀ ; therefore, the above cross gives only hermaphrodites. Of course, if we do not attempt to assume the method of origin of the hermaphrodite, the case may be covered by considering the hermaphrodites pure for this character, while the males would be heterozygous:

$$\female \female \times \female \female = \female \female$$
$$\female \female \times \female \male = \female \female + \female \male$$

COLOR OF SKIN

Colors of grapes are not sharply differentiated, grading from white through many shades of red and purple to black. Because of this wide range, the problem of finding varieties which are pure for certain colors

has been greatly complicated, yet until we are able to find such pure colors and to study their various combinations our knowledge of the color composition of many varieties will probably be only conjectural.

The thousands of seedlings which have been fruited have made possible the formulation of but two general laws: (1) White is a pure color; and (2) white is recessive to both black and red. White, yellow, green, and amber are all considered under the one term and are regarded as being the absence of red and black.

No black variety that has been tested to an extent sufficient to make the results at all conclusive has proved pure for blackness. Some have a factor for red; others seem to contain only black and its absence, white, while still others have both white and red progeny.

In order to simplify the study of red varieties, it has seemed best to divide the seedlings into two shades, the light or medium reds and those ranging from dark red to purple. Those in the second classification are probably red plus either an intensifying factor or various amounts of black. Such a division is somewhat arbitrary and some colors are difficult to place, but, in general, it is a helpful arrangement. Table I gives the result of combining similar colors and shows that the results obtained from crosses of red varieties are as diverse as those from the black. The table includes mainly pure seedlings.

TABLE I.—*Results of crossing grapes of similar colors*

Colors of parental types.	Colors of seedlings.			
	Black.	Purple to dark red.	Medium to light red.	White.
White × white..........................	....	....	....	166
Light red × light red...................	8	6	13	8
Dark red × dark red	38	43	45	42
Black × black.........................	407	49	13	54

Table II illustrates the variation in color composition among most of the varieties given above in the cross black × black and shows the number of varieties which fall in similar groups. From this table it will be seen that 15 black varieties have given only black seedlings, but the number of seedlings is not large enough to be conclusive.

TABLE II.—*Color groups of pure seedlings of black varieties of grapes*

Number of parental varieties.	Colors of seedlings.		
	Black.	Red.	White.
6	52	16	29
6	128	31	....
10	71	....	25
15	132	....	....

It is interesting to note that the parents with only black and white seedlings produce these colors in the ratio of 3 to 1 and that the seedlings of the varieties yielding only black and red are reasonably close to this same ratio.

The results when different colors are combined are given in Table III. The range of color in the seedlings again emphasizes the necessity of knowing more of the color composition of a variety than can be determined from its appearance.

TABLE III.—*Results of crossing grapes of different colors*

Color combinations of parents.[a]	Colors of seedlings.			
	Black.	Purple to dark red.	Medium to light red.	White.
White × dark red [b]	5	44	14	50
White × black	41	3	3	12
Black × dark red	100	52	40	32

[a] Light-red varieties were not used to an extent sufficient to make the results of value.
[b] The reciprocal cross is included in each case.

It would take up altogether too much space to report upon the color of the progeny of all the varieties studied, but a few of the more common ones are given in Table IV, in order to show the wide variation in different varieties of similar color.

TABLE IV.—*Variation in color of pure seedlings of certain varieties of grapes*

Parent.		Pure seedlings.			
Name of variety.	Color.	Black.	Purple to dark red.	Medium to light red.	White.
Agawam	Purple red	1	2		2
Brighton	Dark red	6	5	9	7
Catawba	Purple red	2	4	3	4
Champion	Black	13	1	1	2
Clinton	Black	15			7
Concord	Black	40	6		12
Essex	Purple black to black	4	2	3	
Hartford	Black	4		1	3
Hercules	Black	3	1	1	10
Isabella	Black	8			1
Merrimac	Black	9	3	6	
Nectar	Black	4	5	2	
Ozark	Black	16			
Pearl	White				15
Regal	Dark red			15	5
Worden	Black	4	3		1
Wyoming	Dark red	1	4	2	3

In the ultimate solution of the problems of color inheritance we shall probably be aided in no small degree by those who are studying the subject from the standpoint of the chemistry of the various colors; thus,

Wheldale (8) has isolated two anthocyanins from species of Antirrhinum which produce different shades of red and three flavones for ivory, yellow, and white. Some work has already been done along this line with the grape. Dezani (3) has found two chromogenic substances in white grapes, and several have reported work on the coloring matter of red grapes, but apparently the results are as yet too indefinite to be of much value to the breeder.

INHERITANCE OF QUALITY

At first thought it would seem useless to attempt a study of such an elusive and composite character as quality the interpretation of which depends so much upon the tastes of the observers; yet in the final analysis it is this character which very largely determines whether a seedling is worth saving or must go to the brush pile, and any addition to our knowledge of its inheritance is worth the effort.

Table V shows the rating of the progeny of various parental combinations which run the gamut of quality. Most noticeable is the very low percentage of seedlings whose quality is good or above good even when parents of the highest quality were used. When we consider the ancestral history of these seedlings, these results are not surprising or discouraging. Our American grapes, except for the *V. vinifera* hybrids, are but a step removed from the wild, only a few possessing sufficient quality to make them stand out from the many thousands too poor to be eaten with relish. In breeding from these we are breeding from the topmost point of the species and the effect of the several hundred poor kinds in the immediate ancestry is to pull the seedlings down toward the "level of mediocrity."

TABLE V.—*Inheritance of quality in grapes*

Parental types.		Types of progeny.										
	Best.	Very good to best.	Very good.	Good to very good.	Good.	Total, good to best.	Fair to good.	Fair.	Medium.	Poor.	Total, poor to best.	Percentage of good or better.
Very good to best × very good...				2	9	11	4	7		1	23	48
Very good × very good...		1	7	5	22	35	8	24	3	14	84	41
Very good × good to very good...			2	8	30	40	9	30	2	20	101	40
Very good × good...				3	6	9	3	43	7	23	85	10
Good to very good × good to very good...			1	6	43	50	20	48	6	36	160	31
Good to very good × good...			1		2	3	8	38	2	15	66	4
Good × good...		1	5	3	31	40	18	59	15	31	163	24
Good × fair...				1	3	4	1	6	1	4	16	25
Fair to good × poor...					2	2	5	9	1	23	40	5
Medium × medium...			1	3	18	22	14	54	20	103	213	10
Poor × poor...				1	1	2		7	2	40	51	4
Total number of progeny..		2	17	32	167	218	90	325	59	310	1,002	21

The tendency for the proportion of seedlings of good quality to decrease as we use parents of poorer quality shows clearly the importance of breeding from varieties of only the highest excellence, and even then we must be reconciled to a relatively small percentage of seedlings of good quality.

Practically every grape in the vineyards of the New York Station which ranks high in quality possesses some blood of *V. vinifera*. A moment's consideration of the history of the species shows us the reason for this predominance. European grapes are centuries removed from the wild and have been subjected to a more intense selection than any other fruit; the "level of mediocrity" has been raised to such a point that the species has become a powerful factor in transmitting high quality.

In this connection it is well to speak again of the future that lies ahead of the breeder who will search out and use those varieties of this potent species which blend best with our hardy native kinds. The ages of selection and breeding in Europe have developed varieties of this one species adapted to nearly as wide a range of climate as is covered by all our native species taken together. The proper selection of parents among these should enable us greatly to extend and enrich our viticulture.

A considerable proportion of the seedlings the results of whose crossing are given in Table V are pure seedlings. These have been separated and tabulated in Table VI. Comparing the percentage of those pure seedlings which are good or above good in quality with the percentage of the remaining similar cross-bred seedlings shows an interesting condition. The pure seedlings are uniformly poorer in quality than the crossed seedlings. Is this due to the decrease in vigor which seems to follow selfing, or is there some weightier reason?

TABLE VI.—*Quality of pure seedlings of grapes*

Parental type.	Types of progeny.				
	Good or above good.	Below good.	Total.	Percentage of good or above.	Percentage of crossed seedlings good or above.
Very good × very good....................	14	25	39	36	47
Good to very good × good to very good....	6	26	32	19	34
Good × good............................	33	102	135	24	25
Medium × medium.......................	17	147	164	10	17
Poor × poor............................	0	31	31	0	10
Total.................	70	331	401	17	31

SIZE OF BERRY

In order to economize space it was necessary to plant the seedlings so close together in the test vineyards that the clusters frequently did not reach full and characteristic size. For this reason the size of cluster can not be discussed, although it is an important factor. The size of the berry, on the other hand, is one of the size factors least influenced by environment and season. The data from these vines should be of value and are presented in Table VII.

TABLE VII.—*Inheritance of the size of the grape berry*

Parental types.	Classes of seedlings.						
	Very small.	Small.	Below medium.	Medium.	Above medium.	Large.	Very large.
Large × large..........................	1	2	3	a 28	a 28	19	8
Large b × medium to large c........	1	6	7	56	34	a 67	6
Large × medium......................	......	1	......	a 4	2	2	......
Medium to large × medium to large.	5	34	35	a 103	59	20	4
Medium to large × medium.........	......	20	35	a 57	37	3	2
Medium × medium..................	4	49	39	a 83	38	11	......
Medium to large × small...........	......	a 13	11	12	12	3	......
Medium to small × medium to small.	26	a 35	12	23	7	......	......
Small × small......................	5	a 16	4	5	3	......	......

a Numbers in the bold-face type represent the mode.
b The reciprocal is included in each cross.
c The use of the two terms shows that the berries varied from medium to large in the same variety.

A study of the various crosses which have entered into Table VII has failed to show any indication of purity for size among the varieties studied. Lacking exact measurements for the various sizes, it is not possible to compute an accurate mean, but the relative position of the mean with respect to the mode can be determined by a short study of the table. The wide variation about the mean, even in crosses where both parents were of the same size, prevents the only cross made between extremes of size, medium to large × small, from showing any clear tendency for the F_1 progeny to be intermediate. The steady decrease in the mean and mode as the parental types grow smaller shows clearly the strong tendency for a variety to produce progeny centering around its own size.

FORM OF BERRY

The ovalness of many varieties of *V. vinifera* is so pronounced that some have given this as a species characteristic and have assumed that ovalness in our American grapes was an indication of the presence of blood of this species, an assumption hardly warranted by the facts. The large number of markedly oval varieties among table varieties of *V.*

vinifera, together with the complete or nearly complete loss of this extreme form in hybrids with our American grapes, would lead us to suppose that this pronounced ovalness is perhaps a nearly pure form and that it is either recessive to roundness or else unites with roundness to produce a less pronounced oval. It is this latter type of oval that is referred to in Table VIII showing the inheritance of berry form. The appearance of so many seedlings with round berries in crosses of such oval varieties would tend to strengthen the idea that this is an intermediate form.

Any study of oblateness is made uncertain by the small number of varieties that possess this form. One of the most pronounced is the Goff, a seedling originated at this Station. The behavior of pure seedlings of the Goff grape would seem to indicate that, in this variety at least, oblateness is a pure form and its disappearance when combined with round, as is shown in Table VIII, would seem to show it as recessive to round

TABLE VIII.—*Inheritance of form of the grape berry*

Parental types.	Types of progeny.						
	Oblate.	Slightly oblate.	Oblate to round.	Round.	Round to oval.	Slightly oval.	Oval.
Oval × oval		1	1	*a* 15	7	2	11
Oval × round to oval				*a* 56	10	15	10
Round to oval × round to oval	3	3	10	*a* 129	30	25	56
Round × oval				*a* 15	1	6	3
Round × round to oval		1	2	*a* 100	9	14	1
Round × round	10	17	22	*a* 333	34	24	17
Round to oval × round to oblate	3		1	*a* 17	2	2	1
Round × round to oblate		4	2	*a* 42	5	2	1
Round to oblate × round to oblate	3		1	*a* 24	2	1	8
Round × oblate				7			
Oblate × oblate	*a* 15			1			

a Numbers in bold-face type represent the mode.

From a study of Table VIII it is seen that the mean would be more nearly coincident with the mode in each cross than was the case in Table VII. This shows clearly the strong tendency for roundness to obscure both oval and oblate.

SEASON OF RIPENING

The period of ripening of a variety depends so much upon the vigor of the vine, the season, cultural methods, and environmental conditions that no very accurate data can be presented. In one year all varieties may be 10 days earlier than normally, while in another year early varieties

may be unusually early; but a cold, wet period late in September and early in October may cause the late varieties to be unusually late. These variations are minimized when the records extend over a number of years. The ripening dates of the seedlings are usually taken for at least three years—not long enough, but much better than if taken for a single year.

In Table IX the ripening season extends approximately through the months of September and October. The first two periods cover about 15 days each, the next two about 10 days each, while the length of the last period is usually fixed by the first killing frosts.

TABLE IX.—*Effect of heredity on season of ripening of grapes*

Parental types with reference to ripening season.	Ripening periods of progeny.					
	Approximate mean.	Very early.	Early.	Early midseason.	Midseason.	Late.
Early × early....................	Sept. 23	8	*a* 30	20	......	......
Early × early to midseason *b*......	Sept. 22	13	*a* 46	18	......	......
Early to midseason × early to midseason........................	Sept. 27	7	*a* 46	42	7	1
Early × midseason..............	Sept. 28	......	20	*a* 22	......	......
Early to midseason × midseason..	Sept. 26	21	*a* 126	100	8	......
Midseason × midseason...........	Oct. 1	11	165	*a* 244	49	......
Early × late....................	...do.....	......	8	8	......	......
Early to midseason × late........	Sept. 27	2	*a* 9	2	......	3
Midseason × late................	Oct. 4	3	20	*a* 27	18	6
Late × late.....................	Oct. 7	......	10	*a* 104	19	14

a Numbers in boldface type represent the mode.
b Each cross includes also the reciprocal.

As would be expected, Table IX fails to show purity or dominance for any one season, but it does show, both in the mode and in the approximate mean, the extent to which the season of the parent influences the offspring. A study of the varieties which enter into the table has failed to show results at all different from those of the group in which they fall.

NEW VARIETIES FROM EARLIER CROSSES

The results of the first 20 years of work were anything but encouraging. Now, however, there is tangible evidence that progress is being made. A vineyard of 1,500 seedlings bred from 1898 to 1903 has by a process of vigorous selection decreased to less than 75 vines, but among this number are several that seem very promising. Five of these have already proved so desirable both at Geneva and in a test vineyard at the Station's Vineyard Laboratory at Fredonia, N. Y., that in the fall of 1914 it was decided to give them names and place them in the hands of the nurserymen.

SUMMARY

In the last 25 years, during which time nearly 10,000 seedlings have been grown, various changes in the methods used at the Geneva Experiment Station have been made as the knowledge of breeding laws has been extended.

Results have compelled the belief that improved varieties of grapes will not be produced to any extent until the fundamental laws of heredity are understood. The present aim is to discover these laws.

The work is now progressing mainly along two lines: (1) The determination of the breeding possibilities of varieties of grapes and (2) the study and interpretation of breeding phenomena.

Nearly 200 varieties of grapes have been used in the breeding work.

Much of the value of the early work was lost by growing too few seedlings of each cross.

Recently *Vitis vinifera* has been used to a considerable extent in hybridization.

The usual method of emasculation has been ineffective in a few cases and may be open to criticism.

One of the surprises in the study of grape varieties was the failure of many commercial sorts to transmit desirable qualities.

In order to study grape varieties, nearly 3,000 selfed, or pure, seedlings have been grown. These are uniformly lacking in vigor.

The inheritance of those grape characters which have sufficient data available has been discussed in this paper.

Of the two types of stamens, reflexed and upright, the first is correlated with complete, or nearly complete, self-sterility, the second with self-fertility.

Self-sterility is probably caused by impotent pollen. It exists in varying degrees and depends to some extent upon the condition of the vine and environmental factors.

Self-sterile varieties of grapes being undesirable from a horticultural standpoint, can we eliminate those with reflexed stamens? The following crosses have given upright and reflexed stamens in the indicated ratios:

$$U \times U = 4.3 \; U:1R$$
$$R \times R = 1.2 \; U:1R$$
$$R \times U = 1 \; U:1R$$
$$U \times R = ?$$

Breeding from the upright varieties only will decrease but not eliminate the seedlings with reflexed stamens.

The following seem to be the results secured in the study of sex inheritance:

Hermaphrodite female $\times$ hermaphrodite male = all hermaphrodites.

Hermaphrodite female $\times$ pure male = $\frac{1}{2}$ hermaphrodites + $\frac{1}{2}$ males.

Two general laws have been formulated with regard to color of the skin: (1) White is a pure color; and (2) it is recessive to both black and red.

No black variety has proved pure for blackness. Some contain white, others white and red. Red varieties are equally diverse.

The colors of pure seedlings of certain varieties show wide variation, even when derived from varieties of similar color.

In the inheritance of quality the most noticeable thing is the low percentage of seedlings whose quality is good or above good. This is probably due to the leveling influence of the wild ancestors from which the seedlings are but a step removed.

Most grapes of high quality possess some *V. vinifera* blood. This predominance of high quality is probably due to the intense selection to which the species has been subjected for centuries.

The pure seedlings on the New York Station grounds have been lower in quality than crossed seedlings.

In the inheritance of size of berry there is no indication of dominance of any one size, though there is a tendency for a variety to produce seedlings approaching its own size.

The oval form of many *V. vinifera* hybrids is probably an intermediate between round and a more pronounced oval. Oblate may be a pure form recessive to round.

The season of ripening of the parent influences to a considerable extent the season of the offspring.

A vineyard of 1,500 seedlings bred from 1898 to 1903 has dwindled through selection to less than 75. Of these, 5 have already proved promising enough to be named.

LITERATURE CITED

(1) BEACH, S. A.
    1898. Self-fertility of the grape. N. Y. State Agr. Exp. Sta. Bul. 157, p. 397–441, 3 fig., 5. pl.

(2) BOOTH, N. O.
    1902. Investigations concerning the self-fertility of the grape, 1900–1902. III. A study of grape pollen. N. Y. State Agr. Exp. Sta. Bul. 224, p. 291–302, 1 fig., 6 pl.

(3) DEZANI, Serafino.
    1910. Le sostanze cromogene dell' uva bianca. *In* Staz. Sper. Agr. Ital., v. 43, fasc. 5, p. 428–438. *Abstract in* Jour. Chem. Soc. [London], v. 100, pt. 2, p. 223. 1911.

(4) DORSEY, M. J.
    1914. Pollen development in the grape with special reference to sterility. Minn. Agr. Exp. Sta. Bul. 144, 60 p., 4 pl. Bibliography, p. 50–60.

(5) ———
    1914. Sterility in the grape. *In* Proc. Soc. Hort. Sci., 1913, 10th Ann. Meeting, p. 149–153.

(6) REIMER, F. C., and DETJEN, L. R.
  1914. Breeding rotundifolia grapes. N. C. Agr. Exp. Sta. Tech. Bul. 10, 47 p.,
    19 fig.
(7) SHULL, G. H.
  1911. Reversible sex-mutants in Lychnis dioica. *In* Bot. Gaz., v. 52, no. 5, p.
    329–368, 15 fig. Literature cited, p. 366–368.
(8) WHELDALE, M.
  1914. Our present knowledge of the chemistry of the Mendelian factors for
    flower-colour. *In* Jour. Genetics, v. 4, no. 2, p. 109–129, 5 fig., pl. 7
    (col.). Bibliography, p. 127–129.

ASCOCHYTA CLEMATIDINA, THE CAUSE OF STEM-ROT AND LEAF-SPOT OF CLEMATIS

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INTRODUCTION

The sudden dying of clematis plants has been known for many years, and there has been much speculation as to its cause and prevention. Apparently the disease occurs in both Europe and America wherever the large-flowered kinds of clematis are grown extensively. From published accounts it is clear that the various writers had in mind the same disease, though they ascribed it to different causes. In 1884 Arthur (1)[1] studied a clematis stem-rot which he suspected of being due to the fungus *Phoma clematidis* Sacc. Trelease (16), Comstock (3), Klebahn (7), and others have considered nematodes as the causal agent. In specimens received from Klebahn, Ritzema Bos (2) found nonparasitic nematodes, while in material of his own collection he found the larvæ of a fly, *Phytomyza affinis*, and a species of Pleospora. Prillieux and Delacroix (9) and Morel (8) believed the disease to be of bacterial origin. Sorauer (13) reports a gall-like formation on the stem of *Clematis jackmanni* near the surface of the soil and attributes the death of the affected plants to *Gloeosporium clematidis*. Green (5, p. 284–285) has reported the relative susceptibility of a few varieties of clematis which he grew, but he did not attempt to ascertain the cause of the disease. Except for a preliminary abstract by the writer (4), the primary cause of the clematis disease has heretofore been unknown.

DESCRIPTION OF THE DISEASE

The clematis disease manifests itself differently on the various species and hybrids. On hybrids grown in the field it is a stem-rot, while in the greenhouse, where the cuttings are propagated, it is a leaf-spot as well as a stem-rot. On *Clematis paniculata* the disease takes both forms.

C. paniculata, a type species, is propagated from seed[2] and when grown in uninfected cold frames or greenhouses remains free from disease. Such seedlings are either potted or placed in beds, where they are planted about an inch apart in rows 4 inches apart. In the fall, when these plants

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[1] Reference is made by number to "Literature cited," p. 341–342.

[2] For description of general methods of propagation and of the various species of clematis see the following: Bailey, L. H., ed. Cyclopedia of American Horticulture. ed. 7, v. 1, p. 327–333, fig. 485–492. 1910.
Le Bêle, Jules. The clematises. *In* Garden, v. 53, no. 1388, p. 544–548, illus.; v. 54, no. 1391, p. 39–40, no. 1395, p. 127; no. 1396, p. 138; no. 1399, p. 200–201; no. 1401, p. 240–241. 1898. *Translated from* Bul. Soc. Hort. Sarthe, 1896.

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have made a growth of 8 to 10 inches, the leaf-spot may make its appearance and be thus carried over the winter on the dead leaves or in lesions formed on the vines. If these plants are left in the beds a second season, the fungus may make its appearance early in spring and increase until by midsummer no vine is wholly free from disease. The leaf-spot may first appear either as a mere dot or as a water-soaked area. With the advent of moist warm weather the former usually leads to the latter. On drying the water-soaked spot becomes tan-colored with a red margin. Plate L shows the general appearance of the disease on *C. paniculata*. The older leaves are badly diseased or dead, and the fungus has grown down the petiole to the node, where in time the vine may become girdled. The younger leaves show the early stages of the leaf-spot. The stem shows the lesions, reddish in color, formed at the nodes and on the internodes. Later these take on a gray color. Plate LI illustrates a group of leaves of *C. paniculata* with spots that are zonate, owing to the unequal growth of the fungus under the influence of changes in temperature. The newly formed spot has a dark margin of red tissue and a lighter center. Pycnidia are produced on the diseased leaves. Succulent growing tissues succumb more readily to the disease than do the woody stems. In the latter it may require a month for the fungus to pass a node. Plate LII, figure 1, shows a portion of a vine of *C. paniculata* 44 inches long on which the lower leaves were wilted, while the distal ones were still turgid. The fungus entered through the stub *a*. It girdled the stem and disintegrated the upper roots, leaving the central cylinder as the only means of communication with the healthy roots below. Pure cultures of *Ascochyta clematidina* were obtained from the boundaries of the lesion. Pycnidia were formed on the stem above the ground. In other cases pycnidia have been found on the epidermis, while the tissues underneath were healthy.

Some of the large-flowered kinds of clematis are grown from seed, but in America the majority of those cultivated are hybrids. They are propagated from cuttings taken from rapid-growing, disease-free vines. The cuttings are made in May or June and consist of a single node with the attached leaves and the internode below. They are placed in moist sand and exposed to bottom heat or else grown in forcing frames. In forcing frames the humidity and temperature are usually higher than is found in the average greenhouse. Under these conditions, if the spores are present, a leaf-spot may be formed, and the entire cutting may be killed or the fungus may be halted at the node. The fungus that has been checked may again become active when the cuttings are potted and placed in the greenhouse, or new infections may take place on the leaves. In the fall some of the plants are placed in storage, while others are kept over winter in the greenhouse and the tops used for cuttings. In the following spring both lots are transplanted into the open field and, unlike *C. paniculata*, are not allowed to trail on the ground. Experience has

taught the nurseryman that supported vines, owing to the better venti- lation they receive, do not die as readily as those left on the ground. They make a vigorous growth, and yet when about to bloom they may suddenly die. It is at this stage that the disease first attracts the atten- tion of the nurseryman, though in reality it was on the plants while they were still in the greenhouse and was there overlooked. Plate LIII, figure 1, shows a plant free from leaf-spot, yet girdled by the fungus lurking in the stub *a*, which in ordinary practice is not removed. Plate LIII, figure 2, is a reproduction of another vine of *C. jackmanni* that had many pycnidia of *A. clematidina* on the old stub. After the removal of this stub some of the discolored tissue still remained. The new shoot formed is wilting, and the split stem shows discolored fibrovascular bundles from which *A. clematidina* was isolated. In advanced stages the roots may disintegrate similarly to that shown in Plate LII, figure 1. The spots on the leaves of *C. jackmanni* resemble those found on *C. pani- culata*, *C. recta*, and *C. virginiana*.

ISOLATION OF THE CAUSAL FUNGUS

By previous writers the dying of clematis plants has been assigned to various factors, but none have discovered the primary cause. The dying of the leaves owing to lack of light, the breaking of the vine by strong winds, and injury by nematodes are factors that have been eliminated as primary agencies, while the constant association of *A. clematidina* points to it as the causal organism. The fungus can be readily isolated by the poured-plate method, using the spores from a crushed pycnidium, by the use of sterile leaf tissues, or by the use of free-hand sections of diseased material. The last-named method consists in making free-hand sections under as sterile conditions as possible by sterilizing the outer tissue and the instruments. If such sections show mycelium they are transferred to sterile media. Some have maintained that no mycelium can be seen in the decayed tissue, but the writer has observed in the tissues 3 to 5 mm. from the boundaries of the lesions mycelium which in plate cultures proved to be that of the causal organism, *A. clematidina*.

A. clematidina grows well on the media generally used in the laboratory. It grows at about the same rate on nutrient glucose agar, oatmeal agar, bean pods or stems, moist oats, and corn meal, producing pycnidia in five to seven days. These pycnidia may show a pink tinge at first and later turn brown. The fungus grows less vigorously on corn-meal agar, potato agar, starch agar, sugar-beet plugs, apple twigs, and sterile raw carrot. Oatmeal and starch agar are at first turned green, but later take on a brown color. On starch agar the mycelium penetrates the medium and forms chlamydospores, as shown in Plate LII, figure 1. These are thick-walled, green-brown bodies filled with oil globules. When placed in water, they germinate readily.

INOCULATION EXPERIMENTS

To prove the pathogenicity of *A. clematidina*, mycelium from pure cultures was inoculated into stems of *C. paniculata* and *C. jackmanni*. In all cases lesions were produced, while the checks remained normal. From such lesions the fungus was reisolated, and, when again inoculated into either host, typical lesions were produced. In all, four sets of inoculation experiments were carried out at various times, making from 3 to 10 inoculations on each of 32 plants. Inoculations on succulent stems caused the vines to wilt in four days, while in one case an inoculation on a woody vine 6 mm. in diameter required 47 days to kill the plant. Pycnidia were produced on all lesions.

Plants of *C. paniculata* were sprayed with sterile water containing spores of *A. clematidina* and then kept under bell jars for two days. On the third day the leaves showed water-soaked spots of various sizes, while the checks, which had been sprayed with sterile water, remained free from disease. To test the effect of temperature on infection, two plants were sprayed with the same spore-laden water and then subjected to different temperatures: 23° C. and 10° C. At the end of five days the plant kept at 23° showed 45 leaf spots, while the plant kept at 10° showed but 1 spot.

Spores placed on the lower surface of the leaves produced more spots than those placed on the upper surface. Typical lesions were also produced on the roots by inoculating them with the mycelium from a pure culture.

The *A. clematidina* isolated from *C. paniculata* was inoculated into growing stems of bean, pea, muskmelon, pumpkin; into stems, petioles, and fruits of eggplant (var. Black Beauty); and into the young shoots of elm. In all cases negative results were obtained. On most of these plants pycnidia were produced on the tissues killed in making the wound, but in no case did the mycelium penetrate the healthy tissues and form a lesion.

TAXONOMY OF THE FUNGUS

Arthur (1) observed a species of Phoma, possibly *P. clematidis*, on clematis, but on consulting the original notes made by him it is clear that he had a fungus different from that found by the writer. On but one occasion has *Phoma* sp. been found and that was a saprophyte on the leaf of *C. paniculata*. It was isolated in pure culture, the mycelium inoculated into the stems, and the spores sprayed on leaves, but in no case were lesions or leaf-spots produced.

Saccardo (11) notes *A. clematidina*, *A. vitalbae*, *A. indusiata*, and *A. davidiana* as occurring on various species of clematis, and their chief point of difference is in the size of the spores. The writer has examined the specimens of *A. clematidina* Thümen on *C. virginiana* collected by Mr. J. J. Davis in Wisconsin and distributed in Fungi Columbiani No.

2503; also those of *A. indusiata* Bres. on *C. recta* in Krieger's Fungi Saxonici No. 1189. In both, the spots resemble those found on *C. paniculata* and *C. jackmanni*. In the former the spores are cylindrical 1-septate and hyaline. They measure 8 to 12 by 3.2μ, the average dimensions being 9.5 by 3.2μ. The spores of the latter species are hyaline to honey-colored, somewhat constricted, and measure 12 to 22 by 6.3μ, with an average of 19 by 6μ.

The writer has repeatedly examined the species of Ascochyta on clematis and found it quite variable. The chief difference is in the spores, though sometimes the pycnidia are more deeply immersed than at other times. Plate LIV, figure 1, shows a pycnidium in the leaf tissues of *C. paniculata*. The spores vary in length from 6 to 28μ and in width from 3 to 6μ, but generally they are about 9 to 13 by 3 to 4μ. Plate LII, figure 3, shows the typical spores. The spores are either 1- or 2-celled, rarely 3-celled. Some leaves of *C. jackmanni* collected in the fall of 1914 showed pycnidia having spores as long as 28μ and averaging 18 by 5.7μ. Inoculations with material from cultures obtained by the isolation of single spores showed that this fungus was the same as that usually encountered. The various differences in color shown by the spores disappear when the spores are plated out under control conditions. Considering the variability of the fungus found by the writer, any of the descriptions given for the different species of Ascochyta described on clematis would in general apply to it. Hence, the name selected is the oldest one, *Ascochyta clematidina* Thümen, the description of which is here emended as follows:

Ascochyta clematidina (Thümen).

Ascochyta clematidina Thümen, Pilzfl. Sibir. n. 619, 1884, *in* Sacc. Syll. Fung., v. 3, p. 396.

On stems and foliage; spots circular, zonate to indefinite; pycnidia (on leaves mostly epigenous, sometimes hypogenous) tan to dark brown, scattered to gregarious, globose to subovoid, immersed, then erumpent, ostiolate, averaging 120μ in diameter; spores variable, subellipsoidal to cylindrical, 1- or 2-celled, septa more or less medial, sometimes constricted, hyaline to dilute honey or olive color, often guttulate, 6 to 28 by 3 to 6.4μ, usually 9 to 13 by 3 to 4μ; exuded spore mass honey-colored, sometimes pink.

On living leaves and stems of *Clematis paniculata*, *C. virginiana*, and the hybrids *C. hendersoni*, *C. henryi*, *C. jackmanni*, *C. ramona*, *C. Duchess of Edinburg*, *C. Mme. Baron Veillard*, and *C. Mad. Édouard André*. According to Von Thümen, it occurs also on living leaves of *C. glauca*. As yet no perfect form of *A. clematidina* has positively been found.

CONTROL EXPERIMENTS IN 1913

In 1913 some 2-year-old plants of *C. paniculata* that had made a dense, matted growth of tangled vines were badly diseased, while a bed of seedlings next to them was free from disease. In an attempt to save the 2-year-old plants, they were cut back to a length of 4 to 6 inches and then sprayed with Bordeaux mixture on July 21. A small area was left unpruned and unsprayed as a check. By October 17 the seedlings,

which had made a growth of 8 to 10 inches, showed an occasional leaf spot. The pruned-and-sprayed plot produced an excellent growth, but had some leaf-spot and a few girdled vines. The check showed many dead plants, and none of the living ones were entirely free from disease.

CONTROL EXPERIMENTS IN 1914

A writer in Garden (10) states that Bordeaux mixture, when applied to diseased clematis plants, was of no benefit in checking the disease. In 1914, spraying experiments were carried out by the writer on 18 rows (about 300 feet long) of plants of *C. jackmanni*, half of which were sprayed with Bordeaux mixture (4–4–50 formula), while the others were left as checks. Four of these, two checks and two sprayed rows, were pruned on June 12 and 25 in such a manner as to remove the dead stubs of the previous year. Plants from which all of the discolored tissue could not be removed without injury to the entire vine were marked with tags. The rows receiving Bordeaux mixture were sprayed every two weeks. The final examination was made on October 19. No difference could be seen between the sprayed and the check rows either in the amount of leaf spot or the number of dead plants. The same held true for the pruned and unpruned rows. However, there was but little leaf-spot, and it was observed that the dead plants in the pruned rows were invariably plants that had been tagged. No doubt the pruning was done too late in the season to be of any benefit. Sulphur dusted on cuttings in the forcing frames did not check the disease. Plants in the greenhouse sprayed with soap-and-sulphur mixture so as to cover the leaves with a thin film were healthier than the unsprayed plants. These, however, were not carried through the second season, and hence the ultimate results are unknown.

Two long, narrow beds of *C. paniculata* were utilized for spraying and dusting experiments in 1914. Bed 1 consisted of yearling plants untreated in 1913. Bed 2 contained 2-year-old plants pruned and sprayed with Bordeaux mixture in 1913. Both beds were divided into plots 6 by 25 feet in size.

Two plots in each bed were sprayed with Bordeaux mixture six times at intervals of two weeks from May 15 to August 8. On the same dates one plot in each bed was sprayed with a soap-and-sulphur mixture composed of 1 pound of soap, 6 pounds of sulphur, and 15 gallons of water. Two and one-half gallons of the mixture, containing 1 pound of sulphur, were used on each plot at each application. On two plots in bed 1 and one plot in bed 2 the plants were dusted six times with sulphur, using 1 pound to the plot at each application. The remaining eight plots (three in bed 1 and five in bed 2) were left untreated for checks.

As the season advanced, the virulence of the disease increased, becoming quite severe on all three check plots in bed 1 and one check plot in bed

2. On August 8 some of the plots were pruned, thus terminating the main experiment. The effect of the different kinds of treatment up to August 8 is shown in Table I.

TABLE I.—*Results of an experiment on the control of leaf-spot and stem-rot of Clematis paniculata*

Bed No. 1.			Bed No. 2.		
Plot No.	Treatment.	Percentage of plants free from disease.	Plot No.	Treatment.	Percentage of plants free from disease.
1	Bordeaux mixture.....	75	9	Bordeaux mixture.....	60
2	Check..............	2	10	Check..............	2
3	Bordeaux mixture.....	80	11	Sulphur.............	70
4	Check..............	10	12	Check..............	45
5	Sulphur.............	80	13	Soap and sulphur......	100
6	Soap and sulphur......	100	14	Check..............	85
7	Check..............	10	15	.....do..............	85
8	Sulphur.............	65	16	.....do..............	85
			17	Bordeaux mixture......	95

The results are more uniform in bed 1 than in bed 2. This may be due to the treatment of bed 2 the previous year. That there was more disease in plots 9 and 10 than in the other plots of bed 2 may be accounted for by the fact that these two plots were used as checks in 1913, and hence were neither pruned nor sprayed in that year. Plots sprayed with the soap-and-sulphur mixture remained free from leaf-spot and lesions on the stems; hence, their condition is rated at 100 per cent. In rating the other plots the amount of leaf-spot, the number of lesions, and number of girdled plants have all been considered.

On the plots 1, 3, and 17, which were sprayed with Bordeaux mixture and which were not pruned on August 8, the spraying was continued to the end of the season. Check plots 4, 14, and 16 also were left unpruned. On October 19, when the final examination of these plots was made, it was found that in plots 1 and 3 the leaves on the new growth were disease-free and that there was but an occasional dead vine. On check plot 4 half of the newly formed leaves were diseased, and about one-third of the vines were dead. Plots 16 and 17 showed about the same amount of leaf-spot, only an occasional spot. The former, however, showed more lesions on the stems than the latter. Check plots 2, 7, 10, and 12, which had been pruned back to a length of 4 to 6 inches and then given one application of Bordeaux mixture, had but little disease as compared with plot 4, which had received no treatment whatsoever.

INJURIOUS EFFECTS OF SULPHUR ON CLEMATIS PANICULATA

The promising results obtained by Smith (12) in dusting asparagus with sulphur for the control of rust led the writer to try sulphur in con-

trolling the fungus on clematis.   Up to August 8 the results were satis-
factory, and no injury was observed.   Soon after the pruning of August
8 there were several hot, dry days followed by a period of rainy weather,
during which water accumulated at the end of bed 1.   Up to the end of
the season only one plant in plot 8 had sent forth a new shoot.   The
other vines were dead, and the stems at the surface of the ground for
about an inch were discolored.   A particle of soil placed on the tongue
had an acid taste.   According to a test made by Mr. R. F. Keeler, 1 gm. of
this soil is equivalent to 0.5 c. c. of 0.1 N acid, while soil from the adjoin-
ing check plot 7 was neutral.   In check plot 7 a few vines died, owing to
the lack of drainage, but it seems apparent that in the other cases the
injury was caused by sulphur that had washed from the foliage and had
accumulated in the upper layer of soil.   As the season advanced, sulphur
injury was observed in the other treated plots, but in these cases the
injury was localized in areas not larger than 2 feet in diameter.   The
injury began to show on the plots sprayed with the soap-and-sulphur
mixture after nine applications had been made, while in the plots dusted
with sulphur it appeared after six applications had been made.

SOAP AND SULPHUR AS A SPRAY MIXTURE

A mixture of about 1 pound of laundry soap and 6 pounds of sulphur in
15 gallons of water was in common use as a greenhouse spray at the nursery
where the spraying experiments were conducted.   It was used with suc-
cess in the control of leaf-blotch, *Diplocarpon rosae* Wolf, on susceptible
varieties of roses grown in the forcing houses.   Halsted and Kelsey (6)
used Ivory soap at the rate of 1 ounce to 4 gallons of water for spraying
Phlox drummondii and the common verbena attacked by powdery mil-
dew and were able to check it.   Another (15) has shown that soap at
the rate of 1 ounce to 1 gallon of water controlled the mildew and
aphids of roses.   R. E. Smith (12) recommended that, in the absence of
dew, whale-oil soap be sprayed on asparagus tops to hold the sulphur
that is to be dusted over them for the control of the rust.   Spiecker-
mann (14) has shown that weak solutions of soap have a nutritive value
and can be assimilated by the higher fungi.

In order to test the toxic effect of soap, mycelium of *A. clematidina* was
transferred to Petri dishes containing soap agar of different strengths—
viz, 2 per cent agar containing alkali-free Ivory soap in the proportion
of 1 pound to 5, 10, 15, 20, and 40 gallons of the medium.   Fifteen c. c.
of such media were placed in each Petri dish.   When the fungus became
established, the diameter of the culture was measured daily, and the
rate of growth was considered as a measurement for toxicity.   Cultures
on 2 per cent agar and nutrient-glucose agar served as checks.   Table II
gives the averages of growth of four or five cultures on each medium
grown under the same conditions at room temperature.

TABLE II.—*Summary of the data on the toxic effect of soap agar of various strengths on Ascochyta clematidina*

Culture medium.	Number of cultures.	Average diameter of cultures after growth for—									
		3 days.	5 days.	7 days.	9 days.	11 days.	13 days.	15 days.	17 days.	20 days.	23 days.
Soap agar (1 lb. to 5 gals.)	5	Mm. 0	Mm. 0	Mm. 0	Mm. 0	Mm. 0	Mm. 0	Mm. 0	Mm. 0	Mm. 0	Mm. 0
Soap agar (1 lb. to 10 gals.)	5	0	0	0	0	0	0	0	0	0	0
Soap agar (1 lb. to 15 gals.)	4	0	7	9	11	15	18	20	22	25	28
Soap agar (1 lb. to 20 gals.)	5	0	11	15	19	23	26	30	34	39	44
Soap agar (1 lb. to 40 gals.)	4	8	20	25	32	40	44	50	56	67	73
Check, 2 per cent agar	5	10	21	29	40	51	61	70	77	85	.....
Check, nutrient-glucose agar	5	15	29	38	48	60	69	77	.....	.....	.....

Plate LIV, figure 2, shows a culture of *A. clematidina* on soap agar. The concentric ring or oily film about the culture may be 2 to 7 mm. wide. Upon the drying of the medium, crystals of stearic acid are seen only in this region, indicating that an active principle given off by the fungus liberated the fatty acid. The culture shows a green discoloration, which is due to the formation of brown-green, thick-walled chlamydospores. These bodies, as well as the mycelium, are filled with oil globules, while none are found in the 2 per cent agar. These cultural experiments indicate that soap at the strengths in which it is used as a contact insecticide has in itself fungicidal value, as well as being a means of adhesion or suspension for other materials.

METHODS OF CONTROLLING THE FUNGUS

The suggestions here given for controlling *A. clematidina* are based upon the observations and experiments made in the last three years. Greater success can be attained by changing the methods of culture than by spraying. Long experience has taught the nurseryman that there is less disease when the hybrids are supported while growing in the field or in the greenhouse than when they are permitted to trail on the ground. This holds true also for *C. paniculata*, but its selling price does not warrant so much expense for labor. This can be overcome by transplanting the plants from the beds to the open field after the first year, placing them far enough apart to prevent matting. Spraying is beneficial to such plants, but before making such applications it is advisable to remove all diseased leaves and dead vines. Plants so treated are disease-free in the fall. If seedlings are grown in a greenhouse where clematis has never been grown before and are kept away from older diseased beds, they will

remain disease-free. The fungus can live as a saprophyte on dead vines kept out of doors in baskets, and under such conditions it has lived over two winters, producing pycnidia and viable spores in abundance. This indicates that the same beds should not be used for clematis in successive years.

On hybrids the disease is primarily a greenhouse trouble and can be overcome by the use of cuttings made from healthy·plants. A light spraying with the soap-and-sulphur mixture has proved satisfactory in the greenhouse. It could readily be applied also in the forcing frames. Diseased leaves or stubs should be removed as soon as discovered so as to prevent the fungus becoming established in the tissues.

The retail purchaser of clematis can prevent the dying of plants by taking proper simple precautions. The plants should be placed in good soil, well drained and on a sunny exposure. As soon as the new shoots have formed the old vine tissue should be carefully cut away close to the new shoots, removing all traces of the brown, discolored wood in which the fungus is to be found. Proper ventilation is obtained by training the plants to a strong trellis.

SUMMARY

(1) The stem-rot and leaf-spot of clematis is caused by the fungus *Ascochyta clematidina* (Thümen.).

(2) The plants are killed by the growth of the fungus down the petiole into the stems, thus girdling the plant at the node. The stem may be girdled also by the lesions anywhere on the internodes. Dead stubs left on the vines are a means of holding the disease over a period of time. New shoots may be formed below the girdled region, but the downward progress of the fungus ultimately kills the plant if the diseased tissue is not removed.

(3) Overwintering out of doors does not kill the fungus in cultures or on dead vines. Whenever the temperature permits, the fungus resumes its growth.

(4) The fungus is readily isolated and grows well on the media generally employed in the laboratory.

(5) The disease has been successfully produced by inoculating *C. paniculata* and *C. jackmanni* with the mycelium from pure cultures. The fungus has been reisolated from such inoculations, and with it lesions were again produced on other vines similarly treated.

(6) *A. clematidina* is not related to other common species of the genus Ascochyta, for inoculations made in growing stems of bean, pea, muskmelon, pumpkin, eggplant, and the young shoots of elm gave negative results.

(7) Spraying the plants with spores will produce the leaf-spot. More spots are produced when the spores are placed on the lower surface of

the leaf than on the upper. A temperature of 23° C. is more favorable for the production of the leaf-spot than a temperature of 10° C.

(8) The matting of the vines produces a condition most favorable for the spread of the disease. Ventilation can be obtained by supporting the vines or by planting them far enough apart to prevent matting.

(9) On the hybrids the disease can be controlled in the forcing frames or in the greenhouse by the use of sprays. In the field, the spraying of hybrids properly supported is of little benefit.

(10) On *C. paniculata* spraying with a fungicide checks the disease. In the field the removal of diseased leaves and vines before spraying is of practical value in controlling the disease.

(11) Sulphur dusted on *C. paniculata* in large quantities may cause injury.

(12) A mixture of 1 pound of laundry soap and 6 pounds of sulphur to 15 gallons of water, when sprayed on cuttings in the greenhouse or on *C. paniculata* growing in the beds, controlled the disease.

LITERATURE CITED

(1) ARTHUR, J. C.
    1885. Disease of clematis. *In* N. Y. State Agr. Exp. Sta. 3d Ann. Rpt., 1884, p. 383–385, fig. 5.

(2) Bos, J. Ritzema.
    1893. Phytomyza affinis Fall., as a cause of decay in clematis. *In* Insect Life, v. 6, no. 2, p. 92–93.

(3) COMSTOCK, J. H.
    1890. The clematis disease, or nematodes infesting plants. *In* West. N. Y. Hort. Soc. Proc. 35th Ann. Meeting, 1890, p. 7–12.

(4) GLOYER, W. O.
    1914. Stem rot and leaf spot of clematis. *In* Phytopathology, v. 4, no. 1, p. 39.

(5) GREEN, S. B.
    1906. Ornamental trees, shrubs and herbaceous plants in Minnesota. Minn. Agr. Exp. Sta. Bul. 96, p. 241–350, 108 fig.

(6) HALSTED, B. D., and KELSEY, J. A.
    1903. Fungicides and spraying. *In* N. J. Agr. Exp. Sta. 23d Ann. Rpt., [1901]/02, p. 415–417, pl. 10–11.

(7) KLEBAHN, Heinrich.
    1891. Zwei vermutlich durch Nematoden erzeugte Pflanzenkrankheiten. *In* Ztschr. Pflanzenkrank., Bd. 1, p. 321–325.

(8) MOREL, F.
    1903. La maladie noire des clématites. *In* Rev. Hort., ann. 75, no. 15, p. 364–365.

(9) PRILLIEUX, E. E., and DELACROIX, Georges.
    1894. Maladies bacillaires de divers végétaux. *In* Compt. Rend. Acad. Sci. [Paris], v. 118, no. 12, p. 668–671.

(10) S., E.
    1898. Clematises dying. *In* Garden, v. 54, no. 1394, p. 99.

(11) SACCARDO, P. A.
    1884–1906. Sylloge Fungorum . . . v. 3, p. 396, 1884; v. 10, p. 301, 1892; v. 14, p. 942, 1899; v. 18, p. 347–348, 1906. Patavii.

(12) SMITH, R. E.
　　　1906. Further experience in asparagus rust control.　Cal. Agr. Exp. Sta. Bul.
　　　　　172, 21 p., 7 fig.
(13) SORAUER, Paul.
　　　1897. Zur Kenntnis der Clematis-Krankheiten.　*In* Ztschr. Pflanzenkrank.,
　　　　　Bd. 7, Heft 4, p. 255–256.
(14) SPIECKERMANN, Albert.
　　　1912. Die Zersetzung der Fette durch höhere Pilze.　I. Der Abbau des
　　　　　Glycerins und die Aufnahme der Fette in die Pilzzelle.　*In* Ztschr.
　　　　　Untersuch. Nahr. u. Genussmtl., Bd. 23, Heft 7, p. 305–331, 3 pl.
(15) T., J. C.
　　　1898. Soft soap for mildew and insects.　*In* Garden, v. 53, no. 1386, p. 492–493.
(16) TRELEASE, William.
　　　1885. Root-galls caused by worms.　*In* Cult. & Country Gent., v. 50, no.
　　　　　1682, p. 354.

PLATE L

Clematis paniculata: Portion of vine showing the general nature of the leaf-spot.

3 ⌐⌐
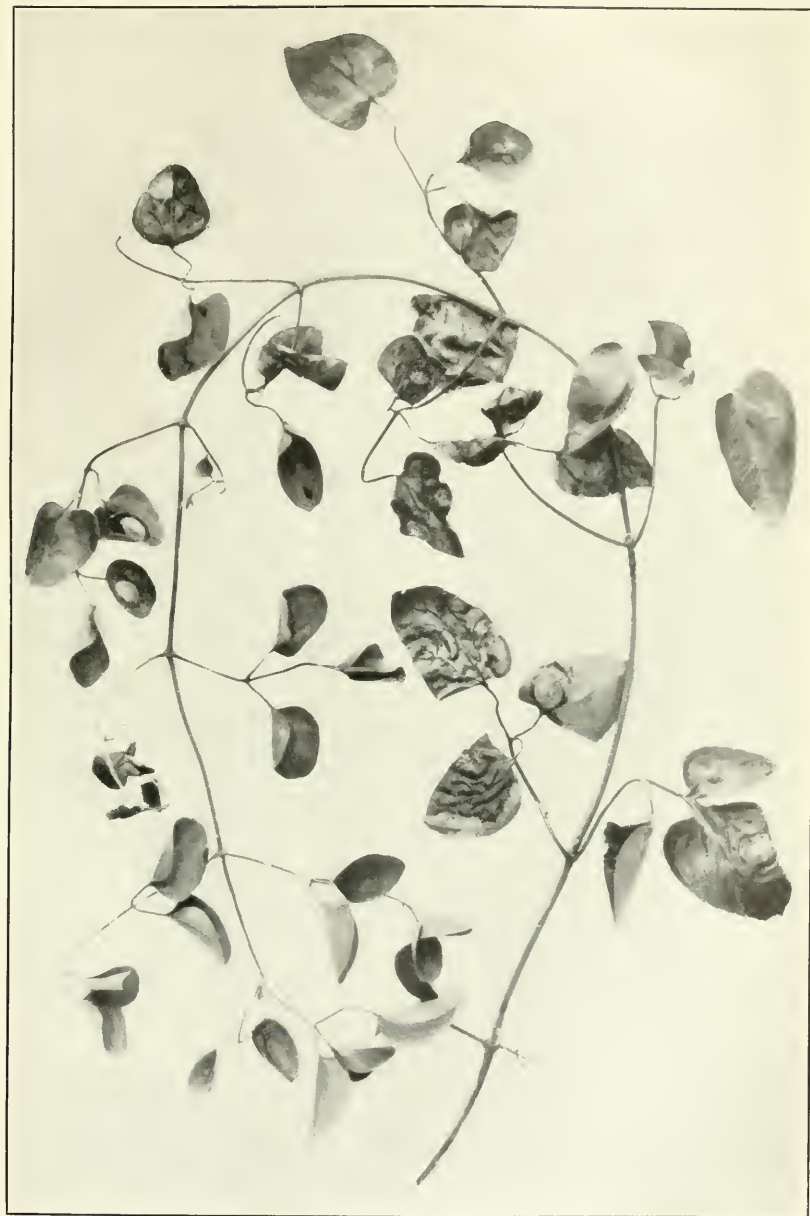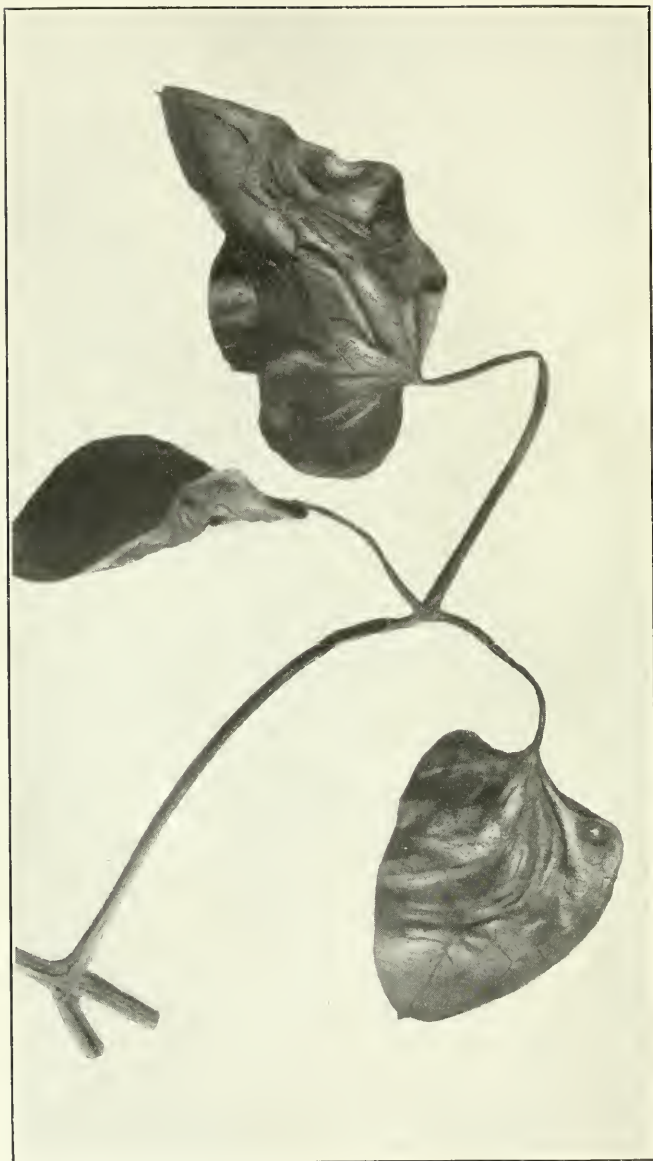
PLATE LI

Clematis paniculata: Group of leaves enlarged to show the zonation and pycnidia of the spots.   One leaf shows the newly formed spot, with its lighter center.

4

Fig. 1.—*Clematis paniculata:* A portion of a vine 44 inches long that showed indications of wilting of the lower leaves while the distal leaves were still turgid. The fungus entered through the stub *a*. In the girdled region the parenchyma of the roots had disintegrated, leaving the central cylinder as the only means of communication with the healthy roots below. *Ascochyta clematidina* was isolated in pure cultures from the boundaries of the lesion.

Fig. 2.—*Ascochyta clematidina:* Chlamydospores as formed on starch agar.

Fig. 3.—*Ascochyta clematidina:* Camera-lucida drawing of spores.
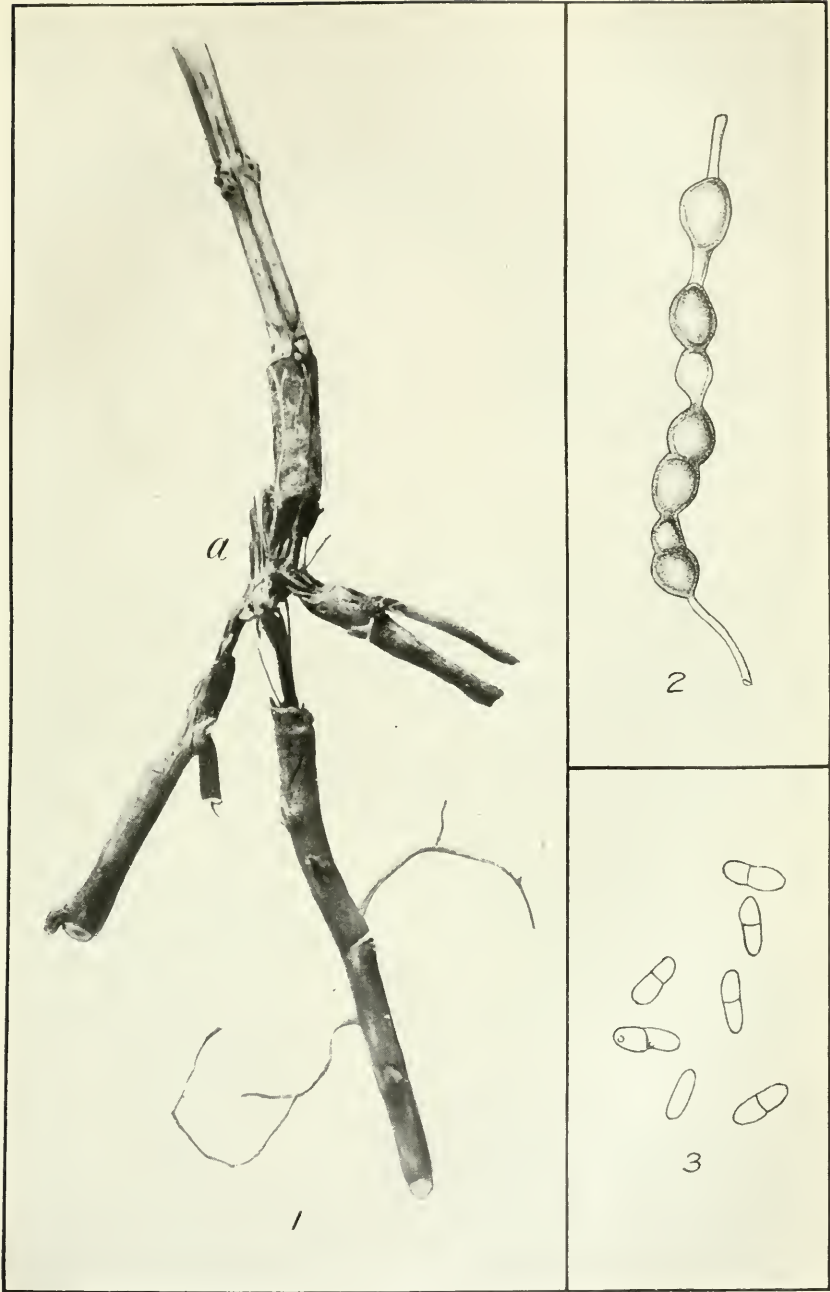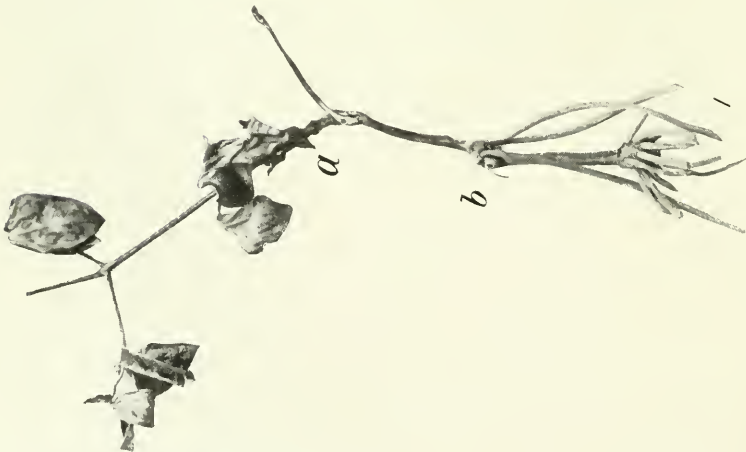
PLATE LIII

PLATE LIII

Fig. 1.—*Clematis jackmanni:* A vine free from leaf-spot that has been girdled by *Ascochyta clematidina* in the region of the previous year's stub *a*.   A new shoot would have been sent forth from an active bud at *b*, but it would have soon died, for the fungus had discolored the vascular bundles beyond this point.   The presence of the fungus was proved by isolating it from the discolored tissue.

Fig. 2.—*Clematis jackmanni:* Plant from which the diseased stub *a* was cut away without removing the discolored tissue.   The leaves were free from leaf-spot and were drying.   The split stem shows the discolored fibrovascular bundles from which the fungus was isolated.

PLATE LIV

Fig. 1.—*Ascochyta clematidina:* Photomicrograph of a pycnidium from stained section of a leaf of *Clematis paniculata.*

Fig. 2.—*Ascochyta clematidina:* Culture growing on agar to which Ivory soap at the rate of 1 pound to 15 gallons of water was added, showing the oily film about the margin of the culture in which the crystals of stearic acid are found.
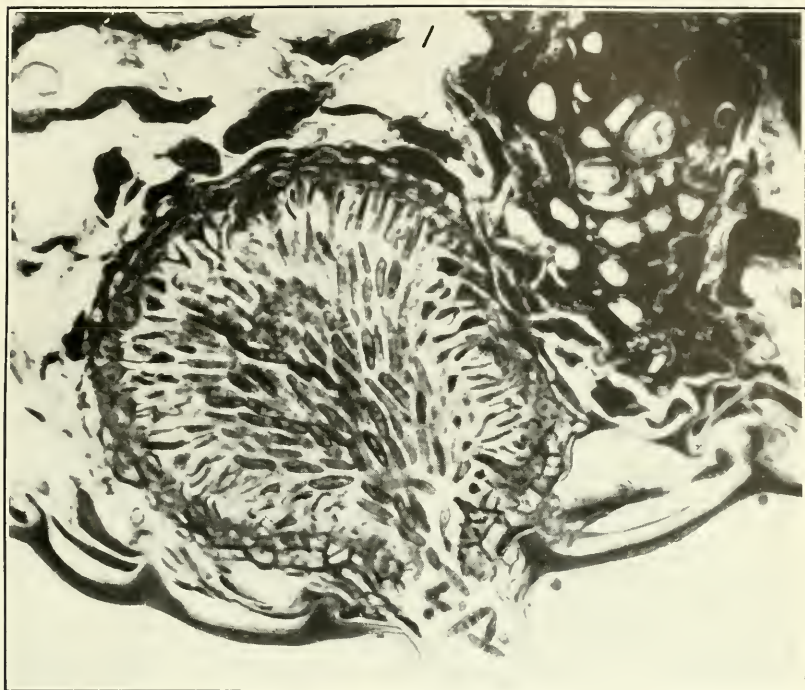
METHODS OF BACTERIAL ANALYSES OF AIR[1]

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INTRODUCTION

This study of methods of making bacterial analyses of the air was undertaken at the New York Agricultural Experiment Station in connection with the problem of determining the relation of the bacterial content of the stable air to the amount of bacterial contamination of milk before it leaves the cow stable. The latter problem forms part of a larger one which has been the subject of investigation at this Station for a number of years and which is now being completed in cooperation with the Illinois Agricultural Experiment Station. Briefly stated, this larger problem is a study of the relative importance of various barn operations in the production of sanitary milk. The work already reported can be found in bulletins of the New York Agricultural Experiment Station (8–11).[2]

In the present investigation two general methods of bacterial air analyses have been tested: One, methods of determining the number of bacteria in a given volume of air; and the other, methods of determining the amount of bacterial precipitation on a known area in a definite period of time. The greater part of the work, however, is devoted to a study of the former technique and involves the determination of the filtering efficiency of two aeroscopes frequently used by investigators and a modification of one of them.

HISTORICAL REVIEW

A brief review of the efforts of bacteriologists to devise satisfactory methods for the bacteriological analysis of the air will show the important stages in the development of the present technique. The methods which have been devised for quantitative purposes generally involve one of the following principles:

(a) Bubbling the air through a liquid, which acts as a filter. The liquid may be either nutritive or nonnutritive. In either case the liquid

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(343)

is examined for bacteria by the usual methods employed for the bacterial analysis of liquids, which include direct microscopical examination, the plating of aliquot portions in a solid nutritive medium, and methods of fractional cultivation in liquid media. When the liquid filter is nutritive, it may be of such a nature as to harden on cooling. In this case the bacteria are allowed to develop in this medium.

(b) Drawing the air through porous solids, which act as filters. These filters may be either soluble or insoluble. According to its nature, the filter is either dissolved or washed in a sterile liquid and the latter analyzed for bacteria as indicated under 1.

(c) The bacteria in the air may be allowed to settle upon the surface of a solid nutrient medium and to develop into colonies wherever they fall. In using this method, a measured quantity of air may be used or the results may be expressed as the number of bacteria falling on a given area in a given period of time. The method may also be varied by using a sterile liquid instead of a solid culture medium, the liquid being afterwards analyzed to determine the number of bacteria present.

It is unnecessary to review the literature of air bacteriology before the time of Petri (20, 21) and Miquel (17, 18) in detail, since these authors have given us very full and complete accounts of the early work. As is well known, this early work had to do very largely with the efforts of Pasteur to convince the adherents of the theory of spontaneous generation that their supposed cases of life without previous life were really due to forms of life already existing in the air. Pasteur used primitive forms of our present methods of bacterial air analysis in several instances. Among these, he tried both soluble and insoluble granular solids as filtering agents, such as guncotton and asbestos. In his later work he used the vacuum-flask method of analysis for rough quantitative work. This method has been further developed by Hansen (7).

Miquel (17, 18, 19) also used several methods of analysis. Among these he tried an "aeroscope" containing a glass plate coated with a sticky mixture of glycerin and glucose which caught the bacteria, mold spores, dust, and the like. Since this technique did not allow a distinction between living and dead organisms, he devised a method of filtering air through water or other liquids and testing the liquids used for bacteria by means of the method of fractional cultivation. When taking samples at a distance from the laboratory, he used solid porous substances, such as glass wool, asbestos, and powdered sodium sulphate, as filtering agents.

Frankland (6) used finely powdered sugar, glass wool, and a mixture of glass wool and glass powder as filtering agents. Hueppe (14) and Straus and Wurtz (28) filtered air through liquefied nutrient gelatin and poured plates from this suspension. Von Sehlen (25) used melted agar in a similar way.

In Petri's (21) first sand filter much coarser sand than that now employed was used and in deeper layers. Two 2.5 cm. layers of a sand which had been heated to redness and which would pass through a 0.5 mm. sieve were supported on the inside of a glass tube on wire-gauze disks. After aspirating a large volume of air through the filter tube by means of an air pump, the sand of each layer (the second acting as a control) was divided between a number of plates and mixed with nutrient gelatin. The number of colonies which developed after incubation was then counted and the number of bacteria originally present calculated from this.

Soper (26, 27) seems to have been the first to use two sand filter tubes in tandem. He washed the sand in sterile water and made his plates from this wash water instead of adding the sand directly to the Petri plates, as former investigators had done. In this way he obtained more transparent plates and probably a more uniform and thorough admixture than had his predecessors.

Winslow (33) compared the results from sand filters made of as coarse sand as that used by Petri with those from filters made of sand of 0.1 to 0.3 mm. in size, with much better results for the latter even when the layer of sand was reduced to 2.5 cm. as compared with 5.0 cm. of the coarser sand.

Weinzirl and Fos (30) were among the first investigators to use a very fine sand of standard size. Among the filtering agents which they employed were sands that passed through sieves with 100 and 150 meshes to the inch and mixtures of the former with powdered silica. They also tried varying the depth of the filtering layer from 0.5 to 2.5 cm. They found that, while the mixture of sand and silica was slightly better than the sand alone, sand which had passed through a 100-mesh sieve was very efficient. They also showed that a 1-cm. layer of sand was as efficient as a deeper layer. The number of tests recorded was small, and in the minds of some there was still a question as to whether or not lanes or pores that would allow the bacteria to pass through might not form in the sand. This point is discussed further on page 349 in connection with the present studies. The above results of Weinzirl and Fos formed the basis for the recommendation of the sand-filtration method by the Committee on Standard Methods for the Examination of Air (2).

One of the recent methods of bacterial air analysis which gave sufficient promise of usefulness to be considered by this committee is that of Rettger (22). This method, which is more fully described on page 348, is a modification of Miquel's method of filtering through distilled water (19) and consists of a device for finely dividing the air as it enters the liquid.

In 1912 a subcommittee (3) gave a report of progress in which they recommended certain modifications in the standard method given in their earlier report. The technique recommended in this later report

was the one which was tested in the present work. Weinzirl and Thomas (31) as members of this subcommittee made a series of comparative tests, using both the new standard method and Rettger's method, securing results slightly favoring the new standard method.

A number of men, other than those mentioned in this review, have contributed to the development of satisfactory methods of bacterial air analysis, among whom are Sedgwick and Tucker (24), William (32), Ficker (5), and others.

The method which has been most used in determining bacterial precipitation is the plate exposure method of Koch (15). He allowed a layer of gelatin to solidify on the surface of a plate in the bottom of a cylinder. The analysis consisted in exposing this layer of gelatin to the air for a known period of time and then allowing the germs to develop on the surface of the medium. Most investigators using the plate exposure method have dispensed with the cylinder, which was designed to prevent side currents of air from affecting the results, and have used Petri plates alone. Koning (16) used a similar principle in some of his analyses. He determined the precipitation per unit area by exposing a known volume of a sterile liquid to the air and analyzing the liquid afterwards.

Koch's method (15) has also been modified so as to relate it to a definite volume of air. Hesse (13) drew air slowly through a long glass tube lined on its inner surface with a layer of gelatin, upon which the bacteria and molds were deposited and allowed to develop.

Winslow (33) modified Hesse's method by substituting two bottles with a layer of gelatin on the bottom of each for the long roll tube. After drawing a liter of air into the system, the bacteria were allowed to settle on the gelatin surface. Weinzirl and Fos (30) agree with Winslow in regarding the method as unsuited for field work.

PRESENT STUDIES OF METHODS

TECHNIQUE

The material to be analyzed was plated in duplicate—or in triplicate in some cases—within one hour after sampling. The medium used was an agar made according to the formula now recommended by the Committee on Standard Methods for Bacterial Milk Analysis (1), except that the acidity was usually between 1.2 and 1.5 per cent normal acid to phenolphthalein. The plates were incubated in a constant-temperature incubator for five days at 18° C. (later 21° C.) and then for two days at 37° C. Except in a few instances, check plates were made which were designed to test out the sterility of the filter tubes, water blanks, and Petri plates. In a few cases where the check plates contained more than the occasional colonies which appear as a result of accidental contaminations, the entire results of the tests were discarded.

The counting of the colonies on the plates was done with the aid of a hand lens. The term "bacteria" used throughout this paper includes yeasts and actinomycetes, since no attempt was made to separate these from the bacteria proper. Molds were noted and recorded separately, but are not given here. In general they were abundant and at times more numerous than the bacteria.

DESCRIPTION OF AEROSCOPES

The term "aeroscope," as used in this paper, indicates an apparatus used to gather bacteria from the air. It does not include the accessories, such as the aspirator bottle and its connections. In the past there has been some variation in the use of the term among different writers. Usually where the principle of filtration through sand was employed, the apparatus has not been called an "aeroscope," while the term is almost always applied to an apparatus in which a liquid is used as the filtering agent.

There seems no good reason for this distinction. Likewise there seems to be very little justification for restricting the word to a part of the apparatus, as is done by Rettger (22).

The aeroscope referred to in this paper (fig. 1) as the "standard" is constructed as follows: A 10-mm. layer of sand which has been passed through a 100-mesh sieve and has been retained by a 200-mesh sieve is supported within a cylindrical glass tube 70 mm. in length and 15 mm. in diameter upon a layer of bolting cloth folded over the end of a rubber stopper. Through a perforation in the stopper there passes a tube 6 mm. in diameter and 40 mm. in length. This tube is attached to the aspirator bottle. The upper end of the cylindrical tube is closed by a perforated rubber stopper through which is passed a glass tube 40 mm. in length and 6 mm. in diameter bent at an angle of 45° in order to prevent precipitation of bacteria or dust particles into the aeroscope.

Fig. 1.—Standard aeroscope.

In using this aeroscope a measured volume of air is filtered through the tube, the sand shaken out into 10 c. c. of sterile water, and aliquot portions of this suspension plated on nutrient agar.

Fig. 2.—Modified standard aeroscope.

The "modified" form (fig. 2) of the standard aeroscope (fig. 1) differs from the standard in that the lower rubber-stopper and bolting-cloth supports are eliminated and the small tube is fused into the larger one. The layer of sand is supported by a layer of cotton resting on the shoulder
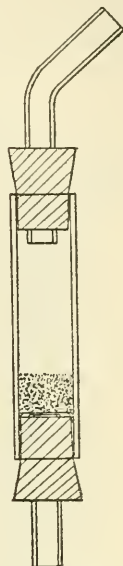
at the junction of the large and small tubes. The upper stopper is replaced by a cork stopper, a construction that permits the aeroscope to be sterilized by dry heat instead of by steam. After this tube was devised it was discovered that Koning (16) had used one of similar construction, although he used a coarser sand in the filter. In this work the sand used in the standard aeroscope and in the modified standard aeroscope was of such a size that it passed a 100-mesh sieve but was retained by a 160-mesh sieve.

Rettger's aeroscope may be described in his own words (22, p. 462–463):

> The entire special apparatus [fig. 3] consists of a glass tube with a small round bulb at one end. The bulb has 8 or 10 small perforations, which serve the purpose of allowing the air to pass through at a rapid rate and yet divide the gas to such an extent that every particle of it is brought into close contact with the filtering fluid. This glass tube or aeroscope is fitted into a small, thick-walled test tube by means of a rubber stopper, which also bears, besides the aeroscope, a short glass tube bent at right angles. The upper end of the aeroscope is bent at an angle of about 45°, in order to prevent bacteria and particles of dust from falling into the open end of the tube, and still permit of the tube being drawn through the stopper without difficulty.

Five c. c. of physiological salt solution are used as the filtering agent. This is plated in aliquot portions, after drawing a measured quantity of air through the aeroscope.

Fig. 3.—Rettger's aeroscope.

EXPERIMENTAL DATA

COMPARISON OF VARIOUS TYPES OF AEROSCOPES

The problem of demonstrating which of two aeroscopes is the more accurate is attended with certain practical difficulties. It is not practicable to set up two aeroscopes side by side and determine which is the more reliable, since the bacterial quality of the air is not uniform enough to make it possible to secure strictly comparable results. For this reason some authors have frankly given up this procedure and have resorted to determinations of the filtering efficiency of each filter independently of the other. In these efficiency tests the aeroscopes are set up in tandem—that is, end to end—so that the air passes through one filter and then through the other. The percentage efficiency of the aeroscope is determined by calculating the percentage of the total number of bacteria that appear in the first aeroscope. Percentages obtained by two different aeroscopes in this way ought not to be compared unless they have been calculated from numbers of approximately equal size. The fairest way of making these comparisons is to carry on the two kinds of tests simultaneously. This was not realized in this work until contradictory results had been obtained by the use of the tests separately.
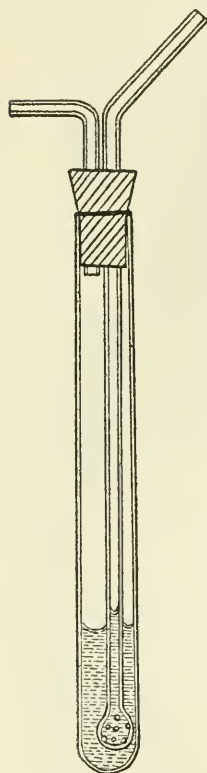
STUDY OF SAND FILTERS IN TANDEM

A series of tests was made, using a sand-filtration method similar in all respects to the standard method except that the rubber stopper with the small tube was omitted from the top of the aeroscope. Two aeroscopes were set up in tandem—i. e., connected end to end by means of a glass tube and two rubber stoppers. In these tests, 5 liters of air were drawn through the filters. No attempt was made to seal the connections leading to the aspirator bottle, but they were made carefully in an attempt to avoid leakage. The plating was done as described on page 346, and in such a way that each plate represented 1 liter of air. In this series of 28 tests the average number of colonies developing per liter was 59 in the first tube (75.9 per cent) and 18.7 in the second tube (24.1 per cent). If three cases, where leakage about the connections between the two aeroscopes evidently occurred, are left out of the calculation, 25 tests gave an average of 50 colonies developing per liter for the first tube (91.4 per cent) and 4.9 per liter for the second tube (8.6 per cent). Later results indicated that slight leakage about the apparently tight stoppers may have influenced even the latter results.

One of the difficulties constantly encountered in air-filtration work is leakage. When this takes place at some point between the filter and the aspirator, the result is that some of the air does not pass through the filter, and therefore the results are low. When, as is probable in the three cases mentioned above, the leakage takes place between the two aeroscopes set up in tandem, the result is that the air goes through only the second filter, making the percentage efficiency of the filter appear lower than it really is.

Another possible condition may have had a marked influence on these results. At times, when sand-filter tubes are sterilized in the autoclave, the sand sticks together to such an extent that cracks appear in it. Such cracked filters are obviously not capable of catching all of the bacteria because of the open pores or lanes through which they may pass. In all of the tests of this series, however, as in all later series, such obviously leaky filters were never used, so that the results given all favor the standard aeroscope to that extent.

TESTS OF RETTGER AEROSCOPES IN TANDEM

A series of 33 tests was run with the Rettger aeroscopes in tandem in order to determine their filtering efficiency. In this work again the difficulty in securing absolutely tight joints was not properly appreciated at first, and rubber connections were used between the two aeroscopes. After sterilization in steam these rubber connections always had a tendency to loosen, and while they were always examined to make sure that they were tight, yet the results indicate that leakage did occur in three cases at least. Seven liters of stable air were drawn through each aeroscope. The aeroscopes contained 5 c. c. of physiological salt solution. One c. c. of the resulting suspension was used in making the plates, agar being

used as the nutrient medium. The average plate count for the first tube in the 33 tests was 18.8, and 2.6 for the second tube (88 per cent efficiency). When the three evident cases of leakage are left out of consideration, the averages become 19.8 colonies per plate for the first tube and 1.5 colonies per plate for the second tube (93 per cent efficiency). In spite of the possible leakage and the fact that the results are all so low that the experimental error due to possible contaminations in plating is high, the results show the Rettger aeroscopes to be fairly efficient.

COMPARATIVE TESTS WITH SAND AND RETTGER AEROSCOPES

Comparative tests were then made between the sand aeroscopes and the Rettger aeroscopes constructed as described (p. 348), but set up singly instead of in tandem. The openings of the two aeroscopes were so placed that they were less than 1 inch apart, and 7 liters of air were drawn through each at the same rate by two aspirator bottles of the same construction. Thus, the filters had an opportunity to obtain air of the same bacterial quality. In order to make the agar plates comparable, 5 c. c. of water were used to wash the sand from the sand filter. One c. c. of each suspension was used in making the plates. The results of these analyses are given in Table I. The averages of the 25 tests are 68 colonies per plate for the sand aeroscope and 30 per plate for the Rettger aeroscope. When calculated on the per liter basis, these averages become 48.6 for the sand aeroscope and 21.5 for the Rettger aeroscope. The same number of colonies per plate was obtained from each aeroscope in six tests. There was 1 excess in favor of the Rettger aeroscope, while 18 excesses were in favor of the sand aeroscope. Six tests were omitted from this table because of bad check plates, but the inclusion of these would not have affected the general results.

TABLE I.—*Comparative counts obtained with the Rettger and an early form of the standard aeroscope*

No. of test.	Agar plate counts.		Counts per liter.		No. of test.	Agar plate counts.		Counts per liter.	
	Rettger.	Standard.	Rettger.	Standard.		Rettger.	Standard.	Rettger.	Standard.
1...........	14	10	10. 0	7. 1	15...........	48	216	34. 3	154. 3
2...........	4	4	2. 8	2. 8	16...........	11	32	7. 8	22. 8
3...........	4	4	2. 8	2. 8	17...........	91	21	65. 0	15. 0
4...........	5	14	3. 5	10. 0	18...........	37	123	26. 4	87. 8
5...........	2	8	1. 4	5. 7	19...........	53	96	36. 4	68. 5
6...........	24	66	17. 1	47. 1	20...........	19	67	13. 5	47. 8
7...........	14	33	10. 0	23. 5	21...........	35	58	25. 0	41. 4
8...........	3	4	2. 1	2. 8	22...........	23	32	16. 4	22. 8
9...........	0	8	0	5. 7	23...........	15	118	10. 7	84. 3
10...........	3	6	2. 1	4. 3	24...........	24	226	17. 1	161. 4
11...........	31	30	22. 1	21. 4	25...........	26	91	18. 6	65. 0
12...........	23	118	16. 4	84. 3					
13...........	9	17	6. 4	12. 1	Average.	30	68	21. 5	48. 6
14...........	237	302	169. 3	215. 7					

Thus far the results were contradictory, since the tests where the aeroscopes were placed in tandem had shown a greater efficiency for the Rettger filters than for the sand filters, while in the duplicate tests of the two types of aeroscopes the sand filters gave better results. Moreover, there were several unsatisfactory features of the tests. Possible leakage had not been perfectly eliminated, the number of bacteria present in the air was so low that slight errors due to contaminations were greatly magnified, and the sterilization of the sand filters with steam produced unsatisfactory results. The latter caused the sand to cake more or less, as already pointed out, and soon injured the rubber stoppers and connections to such an extent that leakage was always a possibility.

At this point a modification of the standard method was tested. A cotton layer sufficiently firm to support the column of sand was inserted just above the lower rubber stopper. The lower rubber stopper was then removed until the aeroscope proper had been sterilized with dry heat. After sterilization the rubber stopper was carefully inserted. Two series of tests of this modified form of aeroscope were made, one of 43 and the other of 38 trials, but the results were so contradictory and inconclusive that they are not given in detail. At this time the modification described and illustrated (fig. 2) was devised.

COMPARISON OF THE EFFICIENCY OF THE MODIFIED STANDARD AND THE STANDARD AEROSCOPE

In comparing the efficiency of the aeroscopes of the standard type with those of the modified type, the aeroscopes were set up in tandem and the tandem couples of each type of filter were used for duplicate analyses. The openings of the aeroscopes were always placed side by side, and similar aspirators were used so that the rate of filtration could be kept the same. The samples of air were all taken in the stable when the cows were in their stalls and during such operations as feeding or milking. This was done because previous experience had shown that the number of bacteria in the air of the stable when it was empty or when everything was quiet was so low that very few colonies appeared on the plates. In every case the sand was shaken out into 10 c. c. of sterile physiological salt solution. One c. c. of the resulting bacterial suspension was added to each plate. Plating was done in duplicate in the first series given in Table II and in triplicate in the second series.

TABLE II.—*Comparative counts obtained with modified standard and standard aeroscopes set up in tandem*

CONNECTIONS NOT SEALED. DUPLICATE PLATING

Test No.	Modified standard aeroscope.			Standard aeroscope.		
	Plate count in first tube.	Plate count in second tube.	Count per liter.	Plate count in first tube.	Plate count in second tube.	Count per liter.
1................	536	1	335	....	6	....
2................	175	68	109	....	31	....
3................	234	0	146	131	13	82
4................	32	1	20	10	0	6
5................	54	0	34	118	1	74
6................	289	1	181	249	4	156
7................	165	4	103	3	6	2
8................	81	4	51	26	7	16
9................	258	42	161	138	189	86
10................	288	46	180	277	33	173
11................	174	4	109	122	19	76
12................	581	7	363	506	12	316
13................	443	5	277	459	99	287
14................	64	2	40	53	12	33
15................	51	7	32	165	22	103
16................	· 140	6	88	191	15	119
Average....	217	12	139	175	29	109

CONNECTIONS SEALED WITH PARAFFIN. TRIPLICATE PLATING

Test No.	Modified standard aeroscope.			Standard aeroscope.		
	Plate count in first tube.	Plate count in second tube.	Count per liter.	Plate count in first tube.	Plate count in second tube.	Count per liter.
1................	185	0. 5	116	116	15	73
2................	511	1. 5	319	582	17	264
3................	382	11. 0	239	378	80	236
4................	500	2. 0	313	485	18	303
5................	382	. 5	239	394	25	246
6................	128	6. 0	80	164	22	103
7................	179	6. 5	112	137	17	86
8................	185	0	116	120	12	75
9................	185	2. 0	116	222	75	139
10................	391	4. 5	244	437	24	273
11................	436	3. 0	273	328	39	205
12................	364	1. 0	228	273	32	171
13................	854	4. 7	534	1, 031	90	644
14................	813	1. 3	508	803	77	502
15................	1, 050	9. 0	656	943	131	589
16................	1, 281	1. 3	801	857	152	536
17................	248	1. 0	155	237	7	148
18................	225	1. 7	141	273	7	171
19................	338	4. 3	211	359	52	224
20................	273	3. 0	171	287	139	179
21................	204	1. 7	128	248	8	155
22................	136	2. 0	85	195	7	122
23................	322	3. 0	201	391	1	244
24................	224	1. 3	140	143	9	89
25................	217	1. 0	136	218	3	136
Average....	400	3. 0	250	384	42	237

In the first series of tests reported in Table II the joints between the two filter tubes were not sealed to prevent leakage, but were carefully examined to discover possible leakage. As the results show (see tests 2, 9, and 10), some leakage probably did occur through the cork stoppers which were used to connect the tubes. For this reason, in the second series of tests all joints which could possibly leak were sealed with paraffin after the apparatus had been sterilized.

The first series of tests (Table II) gave an average of 217 colonies per plate for the first aeroscopes of the tandem pairs in the modified form of aeroscope, with 12 per plate (5.7 per cent) for the second aeroscopes. In comparison with this, the first tubes of the selected (p. 349) standard aeroscopes gave an average of 175 colonies per plate, with 29 colonies per plate (13.2 per cent) for the second aeroscopes of the tandem pairs.

Twenty-five comparative tests in the second series (Table II) under the rigid conditions just described gave an average of 400 colonies per plate for the first aeroscope of the modified form and 3 per plate (0.75 per cent) for the second. The selected standard aeroscopes gave an average of 384 colonies per plate for the first aeroscopes and 42 per plate (about 10 per cent) for the second ones. Thus, while the standard aeroscopes allowed an appreciable number of bacteria to pass through into the second tube, the modified form probably did not allow any bacteria to pass through into the second tube, the small numbers of colonies occurring on the plates being scarcely more than the numbers that appeared on the check plates.

The second series (Table II) shows conclusively that the standard aeroscopes allow bacteria to pass through the sand layer, since all other leakage was cut off by the paraffin seal. It is probable that a more careful selection of those standard aeroscopes in which the layer of sand was least affected by the steam sterilization would have resulted in a higher average efficiency than was found in these tests. On the other hand, if no selection had been made, the results would undoubtedly have shown a much lower average efficiency. The mere fact that such a selection is necessary in order to secure reliable results is of itself a serious weakness in the standard procedure.

The idea that the low numbers on the plates of the second aeroscope in the train of the modified standard aeroscope really represent contaminations is further supported by the fact that an examination was made of the cotton plugs used to support the sand filter in the modified aeroscope. In 25 of the tests, after the sand in the first tube had been poured out, the upper end of the tube that had been in contact with this sand was carefully flamed and the cotton plug blown out into a test tube containing 10 c. c. of sterile water. After a thorough shaking, this bacterial suspension was plated in duplicate in 1 c. c. quantities. In the entire series the average number of colonies that developed per cotton plug was only 50 (5.0 colonies per plate), the highest number

being 132 (13.2 colonies per plate) and the lowest 0. Undoubtedly a large part, if not all, of the bacteria present were really in the sand or clinging to the walls of the aeroscope and were scraped out of the tube with the cotton plug. Even if they were all present in the cotton, they represent less than 1 per cent of the total number of bacteria caught.

All of the analyses thus far described were made by the writer. Since it was desirable to know whether another worker would get equally good results with this modified form of aeroscope and also whether the sand filter was equally efficient when a more rapid current of air was passed through it, Mr. Kulp, who had never before had experience with bacterial air analyses, undertook a new series of analyses. In this series of 22 tests, the air was aspirated at the rate of 1 liter per minute or even faster in some cases (7 liters in five minutes), while all of the previous analyses had been made with an aspiration rate of 1 liter in two minutes. Connections were sealed with paraffin, as before. The material was plated on both agar and lactose agar, but only the results of the former will be discussed here. The samples were plated in triplicate, as in the preceding series. The results of this series of tests are given in Table III.

TABLE III.—*Efficiency tests of the modified standard aeroscope*

[Aeroscopes set up in tandem. Aspiration more rapid than in previous tests]

Test No.	Plate count in first tube.	Plate count in second tube.	Count per liter.	Test No.	Plate count in first tube.	Plate count in second tube.	Count per liter.
1	58	6	83	14	56	7	80
2	48	4	69	15	93	11	133
3	29	1	41	16	75	7	107
4	45	13	64	17	60	2	86
5	28	3	40	18	43	2	61
6	138	1	197	19	15	0	21
7	205	2	293	20	66	0	94
8	282	1	403	21	43	1	61
9	94	1	134	22	30	1	43
10	74	2	106	23	43	1	61
11	38	2	54				
12	66	0	94	Average	76	3	105
13	55.	0	79				

From this table it will be seen that the first tube of the modified form of aeroscope gave an average of 76 colonies per plate (96.3 per cent), while the second tube gave 3 colonies per plate (3.7 per cent). The cotton plugs were plated as before, resulting in the development of 36 colonies per plug (3.6 colonies per plate). Since the number of colonies that appeared on the plates made from the second tube was the same as in the previous series (Table II, second series), it was felt that the lower percentage of efficiency in this case was largely the result of the fact that fewer bacteria were present per liter of air.

COMPARISON OF THE EFFICIENCY OF THE MODIFIED STANDARD AND THE RETTGER
AEROSCOPE

Since the previous work with the Rettger aeroscope had not given conclusive results, another series of tests was carried out. As before, two large aspirator bottles were used and 8 liters of air were drawn through each filter at the rate of ½ liter per minute. The aeroscopes were set up in tandem and all joints carefully sealed with paraffin. In this case the suspensions were plated upon two different media, but only those results secured by the use of plain nutrient agar will be discussed at this place, the other results being referred to later. (See p. 363.) Since the results given as plate counts from the two aeroscopes are not comparable because the Rettger suspensions consisted of 5 c. c. and the sand suspensions of 10 c. c. of physiological salt solution, they have also been computed per liter of air. The latter figures are given in the fourth and seventh columns of Table IV. In this series of comparisons the Rettger suspensions were plated in duplicate and the sand suspensions in triplicate.

TABLE IV.—*Comparative counts obtained with modified standard and Rettger aeroscopes*

Test No.	Modified standard aeroscope.			Rettger aeroscope.		
	Plate count of first tube.	Plate count of second tube.	Count per liter.	Plate count of first tube.	Plate count of second tube.	Count per liter.
1	171	9. 3	214	35	5. 0	22
2	88	2. 3	110	47	1. 5	29
3	122	4. 0	153	61	2. 5	38
4	125	2. 6	156	59	10. 5	37
5	73	1. 3	91	69	9. 0	43
6	117	2. 0	146	62	19. 0	39
7	91	0	114	27	10. 5	17
8	73	2. 3	91	43	1. 0	27
9	46	1. 6	58	32	7. 0	20
10	48	1. 3	60	22	0	14
11	45	1. 0	56	10	1. 0	6
12	79	1. 0	99	27	3. 0	17
13	115	1. 6	144	27	1. 0	17
14	132	1. 6	165	63	1. 5	39
15	130	2. 6	163	15	15. 0	9
Average	97	2. 3	121	40	5. 8	25
16	51	2. 3	64	69	5. 5	43
17	47	. 6	59	131	. 5	82
18	73	0	91	....	....	....
19	79	1. 0	99	87	0	54
20	68	1. 6	85	74	2. 0	46
21	178	2. 3	223	31	. 5	19
22	81	1. 6	101	44	. 5	28
23	72	. 6	90	15	2. 0	9
24	47	1. 0	59	13	3. 0	8
Average	77	1. 0	97	58	1. 8	36

A break has been made in Table IV because of a slight change which was made in the method of plating the bacterial suspensions from the Rettger tubes. In the first 15 analyses the aeroscope arm through which the air enters was washed out once, just before the bacterial suspension was used for plating, by drawing the suspension up into this arm by suction and then releasing it. This was done because it was realized that there was danger that some bacteria would be lost by clinging to the moist inner surface of this tube. As the counts were made, it became evident that the number of colonies appearing per liter from the Rettger aeroscope was less than the number appearing per liter from the modified sand aeroscope. For this reason, beginning with test No. 16 the rinsing of the entrance arm to the Rettger aeroscope was done more thoroughly (several times). By this means the number of colonies appearing on the Rettger plates was increased, showing that the surmised effect of this moist tube was probably true. This effect of the long entrance tube in reducing the number of bacteria in the water filter is also realized by Rettger, for he states (22, p. 467) that one may expect as high as a 15 per cent error in this way. For this reason he recommends drawing the tube out of the aeroscope (after the steam sterilization and before use) and flaming it in order to make sure that the tube will be dry when used. This was not done in the present series of tests, because it was felt that this manipulation after sterilization introduced too great a chance of accidental contaminations.

The results secured before and after the change of procedure noted were as follows: When the entrance tube was rinsed but once, the Rettger aeroscope gave an average of 25 and the modified standard aeroscope gave 121 colonies per liter. After the rinsing was done more thoroughly, there was still a large discrepancy in results, the Rettger filter giving an average of 36 and the modified sand filter an average of 97 per liter.

In the first 15 tests the average counts obtained from the second tube of each tandem pair was 2.3 colonies per plate for the modified standard aeroscope and 5.8 per plate in the case of the Rettger aeroscope. On the basis of these numbers, the percentage efficiencies calculated for the two aeroscopes are 97.7 and 87.3, respectively. The low percentage efficiency obtained with the Rettger aeroscope is somewhat misleading, owing to the fact that in this case the total numbers obtained by both aeroscopes of the tandem pair are low. In the last nine tests the percentage efficiencies were 98.8 for the modified standard aeroscope and 97.1 for the Rettger aeroscope.

COMPARISON OF RETTGER AEROSCOPE WITH THREE TYPES OF THE MODIFIED STANDARD
AEROSCOPE

In order to ascertain, if possible, the reason why the Rettger aero-
scopes had given such decidedly lower results in the previous series
of analyses than the sand filters, another series of analyses was made.
From the data already secured it did not seem probable that the lower
figures could be explained entirely by loss due to the clinging of bacteria
to the entrance tube. For that reason a series of tests was planned in
order to discover whether the difference in the size of the entrance tubes
had any effect on the number of bacteria caught in the filters. Four
types of aeroscopes were used: (1) The Rettger aeroscope; (2) the
modified standard aeroscope used in the preceding experiments; (3) an
aeroscope of the same type as No. 2 except that the entrance tube was
of the same diameter and length as the one used in the Rettger aero-
scope; and (4) an aeroscope of the same type as the second except that
the top was left wide open when the air was being drawn through it,
precipitation of bacteria and dust being prevented by means of a screen
placed above it.

Ten liters of air were drawn through each aeroscope at the rate of ½
liter per minute. Five c. c. of sterile water was used in the Rettger
aeroscope as the filtering agent and the same quantity of sterile water
was used in making the bacterial suspensions of the sand in the other
aeroscopes. Plating was done in triplicate in the usual manner, the
entrance tubes to the Rettger aeroscopes being more thoroughly rinsed
(about 12 times) than had previously been done. The results of 21 such
comparative tests computed on the basis of per liter counts are given
in Table V. The averages of the tests showed that the plates made
from the Rettger aeroscopes (column 1) developed 90 colonies per liter,
those from the modified standard aeroscopes (column 2) developed 90
per liter, those from the modified aeroscopes with the small opening
(column 3) developed 97, while those from the modified aeroscope pro-
tected by a shield (column 4) developed 80 per liter. Individual results
varied greatly, sometimes one aeroscope and sometimes another giving
the highest numbers. From this series of analyses it seems evident that
the size of the opening has little influence upon the number of bacteria
caught by the filters, and it also shows that under some conditions the
Rettger aeroscope may catch as many bacteria as the sand filters. Just
why the Rettger aeroscope should have proved more efficient in the
series of tests given in Table V than in the series given in Table IV is
not evident from the analyses given, unless it was due to the more
thorough rinsing of the inlet tube.

TABLE V.—*Comparative counts obtained with four different types of aeroscopes*

[Bacterial counts given per liter of air]

Test No.	Rettger aeroscope.	Modified standard aeroscope.	Modified standard aeroscope.[a]	Modified standard aeroscope.[b]
1	23	20	19	....
2	67	34	33	....
3	26	99	23	....
4	14	40	12	....
5	14	19	11	....
6	98	265	143	....
7	73	73	305	110
8	52	248	48	129
9	38	75	83	155
10	60	120	107	75
11	171	115	39	34
12	30	31	15	25
13	39	40	91	33
14	73	58	101	56
15	27	121	81	111
16	211	95	192	94
17	116	77	83	87
18	77	79	63	72
19	80	229	291	189
20	133	44	124	126
21	31	24	24	26
22	36	28	48	46
23	65	27	42	32
24	38	14	12	33
25	156	178	188	95
26	41	123	53	50
27	343	94	57	98
Average for 21 tests	90	90	97	80
Average for 27 tests	79	88	85	....

[a] The ordinary inlet tube of this form of aeroscope (see fig. 2) was replaced by an inlet tube of the diameter and length of the inlet tube on the Rettger aeroscope.

[b] No cork or inlet tube was placed in the upper end of these aeroscopes. Precipitation of dust was prevented by means of a shield placed above the opening.

EFFECT OF USE OF LIQUID FILTERS ON COUNT

Since one of the chief differences between the Rettger type of aeroscope and the modified standard aeroscope is in the filter and since there are several possible ways in which the use of a liquid filter might tend to lower the count, further investigations were made. As both distilled water and physiological salt solution had been used in making the tests with the Rettger aeroscopes, series of analyses were made with both of these in which the bacterial suspensions were plated at once and again three hours later. The actual time during which these substances might exert a deleterious influence upon the bacteria was about 30 minutes longer in the case of the Rettger aeroscope than the times given, as it was impossible to make the first plating until about that interval of time had elapsed after first starting the air bubbling through the liquid.

While these tests were being made with the Rettger aeroscope, duplicate tests were run with the modified standard aeroscopes. The bacterial suspensions were plated in this case immediately after being made and

again after an interval of three hours. The detailed results will not be given, since they were somewhat contradictory and bear upon another line of investigation now in progress. In some cases there was a great reduction in numbers of colonies appearing on the plates after the bacterial suspensions had stood for the three hours, in others little or no reduction, and in some cases even an increase. There seemed to be little difference between the action of the distilled water and that of the physiological salt solution.

One interesting result that appeared as the result of these comparative tests was that the Rettger filters in this series of analyses caught nearly though not quite as many bacteria as the sand filters. The exact averages for the 26 tests were 142 colonies per liter for the Rettger aeroscope as compared with 173 for the modified standard aeroscope. Of all analyses given in this paper, 82 give a direct comparison between the Rettger aeroscope and the modified standard aeroscope. Of these 82 comparisons, 49 show larger numbers of bacteria caught by the sand filters, 17 show excesses for the water filter, and 16 give practically the same figures for both. However, if we take only the last 54 of these analyses, omitting the first comparisons, which were not properly carried out in all their details, the showing is more favorable for the Rettger aeroscopes. In these there were 22 excesses for the sand filter, 16 for the liquid filter, and 16 where the results were practically identical.

From these analyses it must be concluded that the Rettger aeroscope is probably very nearly as efficient as the modified standard aeroscope in the hands of an experienced man. There are several things about it, however, that are liable to cause trouble and others that make it less convenient to use. One already mentioned is the possible injurious effect of the liquid used as a filter upon the bacteria before the material can be plated, owing to absorbed gases, unfavorable osmotic action, or other causes making it necessary to complete the plating as quickly as possible after starting the analysis. This one objection makes it inadvisable to use this type of aeroscope for analyses made at a distance from the laboratory. A second difficulty is the need of great care to prevent bacteria from being held on the moist inner surface of the inlet tube. A third is the necessity of steam sterilization, which occasionally loosens the joints about the cork, causing inaccuracies. Since the modified form of aeroscope met all of these difficulties and was cheaper, easier to operate, less likely to break, and more adaptable to field work, it was decided to use this in the investigation to which the work recorded in this paper was preliminary.

COMPARISON OF RESULTS OBTAINED BY DUPLICATE ANALYSES MADE WITH THE SAME TYPE OF FILTER

Inasmuch as the conclusions drawn from data already presented are mainly based upon the averages of from 25 to 30 comparisons, it may be instructive to find out whether such conclusions are justified or whether

they are due to incidental differences in the bacterial quality of the air. Accordingly three series of comparative tests were made, using the same type of filter on each side of the comparison. The first series of 32 such comparisons is recorded in Table VI. In this case two sand filters of the type described on page 351 were used. Six liters of air artificially enriched with dust were drawn through each filter at the same rate. The sand was washed in 5 c. c. of sterile water, and 1 c. c. of the suspension was added to each plate.

TABLE VI.—*Comparative counts obtained with two standard aeroscopes modified to allow dry sterilization*

Test No.	Plate count.		Count per liter of air.	
	Sample.	Duplicate.	Sample.	Duplicate.
1	109	132	91	110
2	358	314	298	262
3	126	357	105	298
4	508	268	423	223
5	323	243	269	203
6	140	89	117	74
7	222	352	185	293
8	194	161	162	134
9	65	73	54	61
10	90	88	75	73
11	126	81	105	68
12	210	186	175	155
13	178	167	148	139
14	318	184	265	153
15	132	157	110	131
16	96	57	80	48
17	710	933	592	778
18	635	602	529	502
19	600	721	500	601
20	862	786	718	655
21	504	212	420	177
22	222	401	185	334
23	384	381	320	318
24	730	886	608	738
25	487	405	406	338
26	370	430	308	358
27	154	194	128	162
28	148	213	123	178
29	20	45	17	38
30	59	25	41	21
31	23	23	19	19
32	93	137	78	114
Average	287	290	239	242

As will be seen from Table VI, 32 tests gave an average of 287 colonies per plate in one sample of air and 290 in the duplicate, or 239 colonies per liter in the sample and 242 in the duplicate. Such close agreement of results, even in series as long as this, are rarely obtained with the technique used. Marked variations occurred in individual cases, however, showing the need of a long series of tests from which to draw conclusions.

DUPLICATE SAMPLING WITH A REVERSIBLE ASPIRATOR

These tests differed from those just given in that the ordinary aspirator bottles were replaced by a reversible aspirator. A Y-connection was placed in the system so that the current of air could be drawn first through one aeroscope and then through the other. Two liters of air were usually drawn through each aeroscope before diverting the current. In this way either 18, 24, or 30 liters of stable air were drawn through each aeroscope, the larger volume being used at the beginning of the series of analyses and the smaller toward the end, when it was discovered that the plates were overcrowded with colonies. Table VII gives the data gathered in this manner. The average numbers of colonies per plate for the two series of 30 tests were 673 and 643. In this comparison the individual results agree a little better than in the previous series, but this is probably partly due to accident and partly to the influence of overcrowded plates. If nine of the comparisons with the most overcrowded plates are left out of the calculation, the averages become 276 for the one set of analyses and 226 for the other. Some individual analyses present wide variations, but the averages show that fairly comparable duplicate results can be secured with this type of aeroscope when used in a series of analyses.

TABLE VII.—*Comparative counts made with two modified standard aeroscopes placed side by side*

Test No.	Number of liters of air.	Plate count.		Count per liter of air.	
		Sample.	Duplicate.	Sample.	Duplicate.
1	24	233	212	49	44
2	24	1,092	1,187	228	247
3	30	485	504	81	84
4	30	434	463	72	77
5	24	341	377	71	79
6	30	204	186	34	31
7	30	12	29	2	5
8	30	379	106	63	18
9	24	1,067	1,281	222	267
10	30	2,121	2,863	354	477
11	24	3,958	1,305	825	272
12	24	1,723	4,445	351	926
13	18	316	329	88	91
14	18	175	190	49	53
15	18	43	118	12	33
16	18	59	28	16	8
17	24	513	561	107	117
18	24	321	102	67	21
19	18	472	198	131	55
20	18	607	1,409	169	391
21	18	868	1,388	241	386
22	18	878	894	244	248
23	18	179	123	50	34
24	18	205	119	57	33
25	18	1,158	674	322	187
26	18	147	283	41	79
27	18	277	146	77	41
28	18	341	404	95	112
29	18	318	115	88	32
30	18	353	154	98	43
Average	....	643	673	144	150

DUPLICATE SAMPLING WITH RETTGER AEROSCOPES SET UP IN TANDEM

A series of 20 duplicate analyses was made with Rettger aeroscopes, using 18-liter bottles as aspirators and filtering 15 liters of stable air through each aeroscope. Five c. c. of sterile water (used instead of physiological salt solution in order to avoid foaming) was used as the filtering agent and 1 c. c. of the suspension was added to each Petri plate. The aeroscopes were also set up in tandem connected by a continuous glass tube. Table VIII gives the results of these analyses in detail.

TABLE VIII.—*Duplicate counts made with Rettger aeroscopes in tandem couples*

Test No.	Sample.			Duplicate.		
	Plate count of first tube.	Plate count of second tube.	Count per liter.	Plate count of first tube.	Plate count of second tube.	Count per liter.
1...............	10	0.6	3	24	0.3	8
2...............	0	.3	0	1	.6	0
3...............	5	11.0	2	6	6.0	2
4...............	15	.6	5	12	.3	4
5...............	31	.0	10	42	.3	14
6...............	5	.6	2	3	.3	1
7...............	3	.6	1	4	.3	1
8...............	12	.6	4	5	.0	2
9...............	29	9.6	10	17	1.3	6
10...............	8	3.6	3	8	2.3	3
11...............	12	.6	4	5	1.0	2
12...............	71	3.0	24	47	3.0	16
13...............	41	3.3	14	20	1.0	7.
14...............	171	52.5	57	57	23.6	19
15...............	35	8.6	12	13	3.6	4
16...............	52	9.3	17	116	3.0	39
17...............	166	3.0	55	52	3.0	17
18...............	51	6.0	17	50	1.0	17
19...............	132	5.0	44	124	3.3	41
20...............	108	2.6	36	129	2.3	43
Average....	48	6.0	16	39	2.8	12

Many of the results show very few colonies per plate, especially where the analyses were made when the cows were out of the stable and everything was quiet. In this respect these figures are unsatisfactory, since low figures greatly magnify contamination errors. The averages of 20 tests gave 48 colonies per plate for the first sample and 39 for the duplicates. The second tubes of each tandem couple gave average counts of 6 (88.8 per cent efficiency) and 2.8 (93.3 per cent efficiency) colonies per plate, respectively.

These three series of comparative tests show clearly that caution must be exercised in accepting the figures of any single comparison as correct. On the other hand, the average of a series of comparative analyses probably gives reliable results.

COMPARISON OF VARIOUS MEDIA FOR BACTERIAL ANALYSIS OF AIR

In the course of this work several comparative series of tests were made to determine whether or not a more suitable medium than ordinary agar might be found. The media so compared with nutrient agar were as follows: (a) Nutrient gelatin, containing the same ingredients as the agar except for the substitution of 10 per cent of gelatin for 1.5 per cent of agar; (b) a gelatin medium with 20 per cent of gelatin; (c) a gelatin medium made with soil extract (4); (d) lactose agar, containing 1 per cent of lactose in addition to the usual ingredients; and (e) asparaginate agar (4), an agar to which only compounds of known chemical composition have been added.

The problem was not studied exhaustively enough to prove that the agar medium used in this work was the best possible medium for air work, but the data gathered did warrant the conclusion that it was better than any medium compared with it. The last two media mentioned above gave only slightly lower results than the nutrient agar, but the three gelatin media gave decidedly lower results, probably due in part to the lower incubation temperature necessary when using them and in part to liquefaction. The 20 per cent gelatin gave the highest results of the gelatin media, owing to diminished liquefaction of the medium.

COMPARISON BETWEEN TWO METHODS OF MEASURING BACTERIAL PRECIPITATION

In the study of the main problem already mentioned, to which this study of technique was preliminary, it was necessary to determine the number of bacteria precipitated upon a given area in a given time. No standard procedure is given for this by the Committee on Standard Methods of Air Analysis, although it seems evident that there should be such a recognized procedure. This determination is usually made by exposing a Petri plate containing solidified agar or gelatin for a given period of time and counting the colonies that develop on the plates after incubation.

It was felt, however, that this method was entirely inadequate, as it does not give a true measure of the number of bacteria falling on the plate. This comes about because of the possibility that a dust particle may carry more than one bacterium. Ordinarily but one colony developes from a dust particle, so that the number of colonies measures the number of bacteria-laden dust particles falling on the plate rather than the number of bacteria falling on the plate.

The method for determining this bacterial precipitation which was tried was to place 500 c. c. of sterile water in a sterilized pail covered with a metal lid. Check samples were then taken and the lid removed for a given length of time. Samples of the water were then taken after thorough agitation and plated as soon as possible. Later it was discovered that Koning (16, p. 251 et seq.) had used milk as the medium

for catching the precipitated bacteria.   Other liquids might be used in the same way.

The satisfactory nature of this technique was demonstrated by the results secured in 34 comparative tests between this technique and the plate-exposure technique.   The results are given in detail in Table IX.

TABLE IX.—*Comparison between the exposed-plate and the exposed-pail methods of measuring bacterial precipitation*

Test No.	Number of colonies developed from bacteria precipitated on 1 sq. cm. in a 5-minute interval.		Ratio.	Test No.	Number of colonies developed from bacteria precipitated on 1 sq. cm. in a 5-minute interval.		Ratio.
	Exposed-plate method.	Exposed-pail method.			Exposed-plate method.	Exposed-pail method.	
1	6.0	56	1:9	23	15.7	216	1:13
2	6.4	58	1:9	24	5.9	81	1:13
3	7.8	261	1:32	25	4.9	36	1:7
4	6.1	51	1:9	26	7.4	17	1:2
5	3.2	28	1:9	27	4.3	13	1:3
6	6.0	34	1:6	28	3.4	23	1:7
a7	8.7	124	1:14	29	11.1	100	1:9
a8	7.8	105	1:13	a30	13.1	216	1:16
9	8.4	63	1:8	31	13.2	30	1:2
10	7.2	45	1:6	32	5.5	32	1:6
11	5.4	62	1:11	33	7.1	28	1:4
12	11.6	109	1:9	34	5.9	53	1:9
13	19.9	250	1:12	35	9.2	53	1:6
14	9.3	36	1:4	36	11.0	68	1:6
15	7.0	75	1:11	37	7.7	96	1:13
16	7.7	49	1:7	38	8.3	58	1:7
17	6.3	81	1:13	a39	11.1	120	1:10
18	5.5	81	1:15	a40	11.6	118	1:10
19	11.0	94	1:9				
20	9.4	184	1:20	Average..	8.1	78.5	1:10
21	10.4	149	1:14				
a22	9.2	199	1:21				

a These analyses were omitted from the averages because of contaminated check plates.

In tests 1–12 and 23–34 the Petri plates were exposed for five minutes, but this length of time was abandoned because of the overcrowding of the plates.   In the other cases, the numbers given were secured by adding the results secured by exposing four different plates consecutively for 1¼ minutes each.   In this way the same time of exposure was secured without overcrowding the plates.

The average result secured in these tests was 8 colonies per sq. cm., secured from a 5-minute exposure where this was determined by exposed Petri plates.   Where the pail method of exposure was used, the similar figures were 78 colonies per sq. cm.   In the most favorable cases the plate-exposure method gave only one half of the numbers secured in the other way, while in the least favorable cases the plate-exposure method gave only 1 colony to 32 colonies which appeared on the plates

made from the pails. Frequently, the colonies that appeared on the plates which had been exposed showed by their very nature that they were of composite origin.

It seems strange that this fundamental weakness of the plate-exposure method has not been properly appreciated by investigators, for it was recognized by Hueppe (14) as long ago as 1891.

This work shows that the figures obtained in all of the air investigations where conclusions are based upon results obtained by the plate-exposure method are not nearly so large as they should have been. The same criticism applies to some of the methods that have been suggested as a means of counting bacteria in air. One of the first to use this faulty principle of regarding bacteria-laden dust particles as equivalent to individual bacteria was Hesse (13). He counted the colonies developing on gelatin after a measured volume of air had been drawn over it in such a way as to catch the dust particles on the gelatin. The idea that the number of colonies developing on the surface of a solid medium after exposure to the air really represents the number of single bacteria deposited seems also to have been held by some of the later investigators, among them being Harrison (12), Russell (23), and Winslow (33).

<div align="center">CONCLUSIONS</div>

It seems reasonable to conclude that the nature of the filters tested had little influence on the results secured in duplicate analyses—that is, those obtained where a sand and a liquid filter were used side by side agreed just as well as those where either two sand filters or two liquid filters were used side by side.

It was found that the particular form of sand-filter aeroscope recommended by the committee on standard methods of bacterial air analysis appointed by the American Public Health Association varied in its filtering efficiency from 50 to 100 per cent, with the average efficiency for two series of tests of 90 and 91.6 per cent. It is believed that the chief cause of error with this form of aeroscope arises from the fact that it is so constructed that it must be sterilized with steam, which causes caking of the sand-filtering layer.

A description is given of a modification of this form of aeroscope, so constructed that it may be sterilized with dry heat. The modified standard aeroscope was found to retain nearly 100 per cent of the bacteria, with little chance of error. It was also found to be cheaper, less breakable, easier to operate, and more adaptable to field work than either the standard sand aeroscope or the aeroscope recommended by Rettger.

The latter can be made to yield excellent results, provided sufficient care is exercised in handling it. Its use, however, is attended with a number of difficulties, among which may be mentioned its tendency to leakage about the rubber stoppers after being sterilized, the foaming of

the liquid during operation, and the tenacity with which the bacteria cling to the inner surface of the moist inlet tube.

The method of determining bacterial precipitation from air by means of exposed Petri plates has been found to be entirely unreliable, as it gives a measure of the number of bacteria-laden dust particles and not a measure of the number of bacteria present. The number of bacteria precipitating upon a given area has been determined by analyzing measured quantities of sterile water which had been exposed to the air for a given length of time in sterile pails. The numbers obtained in this way were from 2 to 32 times higher than those obtained with the plate-exposure method.

LITERATURE CITED

(1) COMMITTEE ON STANDARD METHODS FOR BACTERIAL MILK ANALYSIS.
   1915. Report. *In* Amer. Jour. Pub. Health, v. 5, no. 1, p. 64.
(2) COMMITTEE ON STANDARD METHODS FOR THE EXAMINATION OF AIR.
   1910. Report. *In* Amer. Jour. Pub. Hyg., v. 20 (n. s. v. 6), no. 2, p. 346–360.
(3) ————
   1913. Progress report. *In* Amer. Jour. Pub. Health, v. 3, no. 1, p. 78–86.
(4) CONN, H. J.
   1914. Culture media for use in the plate method of counting soil bacteria. N. Y. State Agr. Exp. Sta. Tech. Bul. 38, 34 p.
(5) FICKER, Martin.
   1896. Zur Methodik der bakteriologischen Luftuntersuchung. *In* Ztschr. Hyg. u. Infectionskrank., Bd. 22, Heft 1, p. 33–52, pl. 1.
(6) FRANKLAND, P. F.
   1888. Methode der bacteriologischen Luftuntersuchung. *In* Ztschr. Hyg., Bd. 3, p. 287–293, 2 fig., pl. 6.
(7) HANSEN, E. C.
   1879–82. Bidrag til Kundskab om hvilke Organismer, der kunne forekomme og leve i Øl og Ølurt. 1. Undersøgelser over de Organismer, som til forskjellige Tider af Aaret findes i Luften i og omkring Carlsberg, og som kunne udvikle sig i Ølurt. *In* Meddel. Carlsberg Lab., Hefte 2, p. 185–235, 1879; Hefte 4, p. 381–454, 2 fig., 1882. French ed. p. 49–75, 197–218.
(8) HARDING, H. A., RUEHLE, G. L., WILSON, J. K., and SMITH, G. A.
   1913. The effect of certain dairy operations upon the germ content of milk. N. Y. State Agr. Exp. Sta. Bul. 365, p. 197–233.
(9) ———— and WILSON, J. K.
   1913. A study of the udder flora of cows. N. Y. State Agr. Exp. Sta. Tech. Bul. 27, 40 p.
(10) ———— ———— and SMITH, G. A.
   1909. Milking machines; effect of method of handling on the germ content of the milk. N. Y. State Agr. Exp. Sta. Bul. 317, p. 253–292, 4 pl.
(11) ———— ———— ————
   1910. The modern milk pail. N. Y. State Agr. Exp. Sta. Bul. 326, p. 249–281, 4 pl.
(12) HARRISON, F. C.
   1897. Report of the bacteriologist. *In* 22d Ann. Rpt. Ontario Agr. Col. and Exp. Farm, 1896, p. 105–116, illus.

(13) HESSE, W.
    1884. Ueber quantitative Bestimmung der in der Luft enthaltenen Mikroorganismen. *In* Mitt. K. Gsndhtsamt. [Germany], Bd. 2, p. 181–207, 3 fig., pl. 11–13 (col.).
(14) HUEPPE, Ferdinand.
    1891. Die Methoden der Bakterien-Forschung . . . Aufl. 5, 495 p., 68 fig., 2 col. pl. Wiesbaden.
(15) KOCH, Robert.
    1881. Zur Untersuchung von pathogenen Organismen. *In* Mitt. K. Gsndhtsamt. [Germany], Bd. 1, p. 1–48.
(16) KONING, C. J.
    1906. Biologische und biochemische Studien über Milch. Vierter Teil: Die Stall-Luft. . . . *In* Milchw. Zentbl., Jahrg. 2, Heft 6, p. 241–263; Heft 7, p. 313–338, 2 pl. Translated by Johs. Kaufmann from Pharm. Weekbl., 1905. (Not seen.)
(17) MIQUEL, P.
    1883. Les Organismes Vivants de l'Atmosphère. 308 p., 86 fig., 2 pl. Paris
(18) ———
    1888. De l'analyse microscopique de l'air au moyen de filtres solubles. *In* Ann. Microg., t. 1, p. 153–167, 5 fig.
(19) ———
    1889? Dixième mémoire sur les poussières organisées de l'air et des eaux. *In* Ann. Observ. Munic. Montsouris [Paris], 1888, p. 413–600, fig. 54–77.
(20) PETRI, R. J.
    1887. Zusammenfassender Bericht über Nachweis und Bestimmung der pflanzlichen Microorganismen in der Luft. *In* Centbl. Bakt. [etc.], Bd. 2, No. 5, p. 113–118; No. 6, 151–158.
(21) ———
    1888. Eine neue Methode Bacterien und Pilzsporen in der Luft nachzuweisen und zu zählen. *In* Ztschr. Hyg., Bd. 3, p. 1–145, pl. 1–4.
(22) RETTGER, L. F.
    1910. A new and improved method of enumerating air bacteria. *In* Jour. Med. Research, v. 22 (n. s. v. 17), no. 3, p. 461–468, 3 fig.
(23) RUSSELL, H. L.
    1897. Tainted or defective milks: their causes and methods of prevention. Wis. Agr. Exp. Sta. Bul. 62, 27 p., 10 fig.
(24) SEDGWICK, W. T., and TUCKER, G. R.
    1888. A new method for the biological examination of air, with a description of an aerobioscope. Paper read and discussed before the National Academy of Sciences at Washington, Apr. 18, 1888. Cited by Tucker (29, p. 164).
(25) SEHLEN, von.
    1884. Studien über Malaria. *In* Fortschr. Med., Bd. 2, No. 18, p. 585–591.
(26) SOPER, G. A.
    1907. The air of the New York subway prior to 1906. *In* Technol. Quart., v. 20, no. 1, p. 58–118, 23 fig.
(27) ———
    1907. Comparison between bacteriological analysis of air by the plate method and by filters. *In* Jour. Infect. Diseases, Sup. 3, p. 82–84.
(28) STRAUS, I., and WURTZ, R.
    1888. Sur un procédé perfectionné d'analyse bacteriologique de l'air. *In* Ann. Inst. Pasteur, ann. 2, no. 4, p. 171–180, 1 fig.

(29) TUCKER, G. R.

    1889. The number and distribution of microorganisms in the air of the Boston City Hospital, with some carbonic acid determinations. *In* 20th Ann. Rpt. State Bd. Health Mass. [1887/88], p. 161–230, 2 fig., 5 pl.

(30) WEINZIRL, John, and Fos, Maud V.

    1910. Bacteriological methods for air analysis. *In* Amer. Jour. Pub. Hyg., v. 20 (n. s. v. 6), no. 3, p. 633–638.

(31) —— and THOMAS, J. B.

    1913. A comparison between the sand filtration method and the bubbling method for enumerating bacteria in air. *In* Amer. Jour. Pub. Health, v. 3, no. 2, p. 171–174.

(32) WILLIAM, N.

    1893. Versuche über die Verbreitung der Cholerabacillen durch Luftströme. *In* Ztschr. Hyg. u. Infectionskrank., Bd. 15, p. 166–178, 2 fig.

(33) WINSLOW, C.-E. A.

    1908. A new method of enumerating bacteria. *In* Science, n. s. v. 28, no. 705, p. 28–31, 2 fig.

WALLROTHIELLA ARCEUTHOBII

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INTRODUCTION

The fungus *Wallrothiella arceuthobii* [1] has the distinction of causing the only disease of the leafless mistletoes so far described. Comparatively few botanists have seen this interesting fungus either in nature or in museums, and all reference to it has been omitted from works dealing strictly with plant diseases. The fungus was apparently never collected again from the region where it was originally discovered, and no record exists of preserved material from the place of its second discovery. The discovery of the fungus by the writer in the Northwest adds a new interest to its study, especially since it is found to be so abundant as to have some economic significance.

Owing to the fact that the fungus has only been reported twice and from widely separated stations, its literature is very meager. It was originally discovered by Peck,[2] who published a short account of it under the following name and description:

Sphaeria arceuthobii, n. sp.

Perithecia small, densely caespitose, oblong or cylindrical, very obtuse, shining black; asci subclavate, fugacious; spores crowded, globose, colorless, .00016″ in diameter.

Capsules of *Arceuthobium pusillum*. Forestburgh. Sept. (Plate I, figs. 10–14).

It forms little black tufts, crowning the fruit at the tips of the stems and branches. I have not seen it on the staminate plant. I am not fully satisfied that the generic reference is correct, as the perithecia seem to be mouthless. It is interesting to observe the extent to which parasitism prevails. The Arceuthobium is a parasite on the spruce, this fungus is parasitic on the Arceuthobium, and in a few instances a third parasite, a minute white mold, was seen on the perithecia of the fungus.

The second discovery of the fungus was by Wheeler [3] in the Upper Peninsula of Michigan, who reports it as follows:

I found that the mistletoe was also attacked by a fungous parasite, which must have a tendency to check the spread of this pest. Each fruit is attacked at its apex by the fungus *Wallrothiella arceuthobii*, Peck, and, of course, destroyed.

Both Peck and Wheeler published some excellent drawings of the fungus, from which a very good idea of the character of the disease may be obtained.

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[1] If the system of classification of Engler and Prantl is employed, the fungus would be referred to Rosellinia. (Engler, Adolf, and Prantl, K. A. E. Die natürlichen Pflanzenfamilien. T. 1, Abt. 1, p. 394, 400, 404, fig. 258, A, B. Leipzig, 1897.)

[2] Peck, C. H. Report of the botanist. *In* 27th Ann. Rpt. N. Y. State Mus. Nat. Hist. 1873, p. 111, pl. 1, fig. 10–14. 1875.

[3] Wheeler, C. F. The geology and botany of the Upper Peninsula experiment farm. *In* Mich. Agr. Exp. Sta. Bul. 186, p. 27–28, 4 pl. 1900.

HOSTS

The interest arising from the remarkable habit of this fungus of attacking and destroying the immature fruits of *Razoumofskya pusilla* (Peck) Kuntze (*Arceuthobium pusillum* Peck) on the eastern black spruce (*Picea mariana*) has led the writer to search most diligently for it on western species of Razoumofskya. The fungus was not found in any region of the Great Lakes States, although the mistletoe of the spruce was abundant and much material was examined. In the West the search has been more successful. The fungus was first collected on *Razoumofskya douglasii* Englm. (Pl. LV, fig. 1) in the vicinity of Como Lake in the Bitterroot Mountains, Montana. The only tree found bearing infected plants stood in a clump of "left-overs" on a cutting area of the Latchem Lumber Co. The mistletoe is so very abundant in this region and suppresses the Douglas fir (*Pseudotsuga taxifolia*) to such an extent that this tree, according to Supervisor White, of the Bitterroot National Forest, is sometimes omitted altogether from the estimate of the prospective cut. Practically the same conditions prevail throughout the entire Bitterroot and Missoula River Valleys and adjacent regions. It has always been a rule of the writer, in regions of a heavy infection by *R. douglasii*, to look for the small, closely related mistletoe designated by Engelmann "*Razoumofskya douglasii*, var. *abietina*," on *Abies grandis* and *A. lasiocarpa*. Experience has shown that this mistletoe is more likely to occur when its hosts are in the vicinity of the Douglas-fir mistletoe. Whatever conclusions may be drawn from this as to the probable relationship of the mistletoe on *Abies* spp. with the one on *Pseudotsuga* spp., the surmises as to the presence of the former were correct in the present instance.

The mistletoe was discovered once on *A. grandis* by the large spreading or upright brooms. The tree, standing not more than 100 feet from a Douglas fir which supported three immense brooms of *R. douglasii*,[1] was felled and the mistletoe plants carefully examined. A few of the plants, which were pistillate, were found to be infected by *W. arceuthobii* (Pl. LV, fig. 2). On another portion of the same area the fungus was discovered on a few plants (Pl. LV, fig. 3) growing deep within a large broom on *A. lasiocarpa*. Two other mistletoe-infected trees of this species were cut, all that were found in the region, but the parasite was not attacked by the fungus. A search in other sections of the Bitterroot Valley resulted in finding the mistletoe on the grand and alpine firs, but the fungus was not present. At the head of Rattlesnake Creek, a small stream flowing into the Missoula River at Missoula, the fungus was discovered on a small mistletoe (Pl. LV, fig. 4) growing on *Picea engelmanni*. This is the first report of a mistletoe occurring on a spruce in the North-

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[1] The brooms caused by this mistletoe are sometimes very large and frequently cause the death of the entire crown above. The infections of no other mistletoe initiate greater and more frequent brooming than do those of *R. douglasii*. For this reason it is one of the most serious parasites of the entire genus.

west. It is apparently the same as that described by Engelmann on spruce from the Sierra Blanca Mountains in northern Arizona under the name "*R. douglasii*, var. *microcarpa*." A single collection of what is apparently the same mistletoe on spruce was made from a recently fallen tree on a cutting area near Laclede, Idaho. The parasite was, however, in a healthy condition. The Douglas-fir mistletoe was found in each of the two last-named regions. The fungus material in all the foregoing cases was very scanty. It is believed, however, that a more protracted search will result in finding the fungus more abundantly on the Douglas-fir mistletoe.

In order to test the ability of the fungus to attack other mistletoes, infected plants of *R. douglasii* were bound in contact with pistillate plants of *R. americana*, the mistletoe of the lodgepole pine (*Pinus murrayana*). To prevent accident, the experiments were protected by binding cheesecloth loosely about the stem supporting the mistletoe, completely inclosing the plants but not interfering with their vital functions. In all, four such experiments were made during the month of October, 1913. Since pollination had already taken place in the early spring, it was inferred that the fruits of the lodgepole-pine mistletoe would mature normally if infection did not occur. In order to have fully mature plants on the same stem for purposes of comparison, small tufts just below the inclosed ones were shielded by a circular piece of thick white cloth tied just above the tuft and hanging down in the form of a loose umbrella. This would not prohibit the circulating spores from coming up under the shield, provided they escaped from the cheesecloth net, but would lessen the chances of inoculation. Furthermore, owing to ascending air currents, spores of fungi usually travel upward or at least not directly downward when starting from an elevated point. Other experiments were initiated on the lodgepole-pine mistletoe by crushing in water a number of mature perithecia and thoroughly spraying the mixture containing spores over a few pistillate plants. To prevent the plants being knocked off during the winter, they were also protected by cheesecloth. These experiments were visited in the latter part of November, 1914. The results were positive. As shown in Plate LV, figure 5, not only had the fruits of the lodgepole-pine mistletoe which were inclosed with the infected plants from the Douglas fir become infected but very thoroughly so. Every fruit bore at its apex the little shiny black tufts of the perithecia of the fungus. One fruit shown to the right in the middle figure of the sprayed plants seemed to have escaped early infection and to have attained nearly a normal size, but, nevertheless, succumbed to the parasite. The tufts of mistletoe just below the infected ones, which were shielded from above, did not become infected and produced normal mature seeds, which were being expelled at the time the experiments were discontinued. The perithecia of the fungus on

R. americana contained mature spores; hence, the life cycle of the fungus in the seed capsule is complete in the fall of the second year coincident with the time required for the ripening of the seeds of the host.

Soon after the conclusion of the experiments, a swamp area in the Kaniksu National Forest, Idaho, scatteringly timbered by lodgepole pine, was visited. The trees were heavily infected with the mistletoes characteristic of this tree. A close examination of the mistletoe plants showed them to be uniformly attacked by the fungus throughout the entire area. So abundant was the fungus that very few of the pistillate plants on any of the trees had escaped attack. This area has been a fruitful source of investigation and a number of important facts have been gathered.

SIGNIFICANCE OF THE FUNGUS TO THE TAXONOMY OF ITS HOSTS

The hosts of *W. arceuthobii*, so far as known at present, are as follows:

Razoumofskya pusilla (Peck) Kuntze on *Picea mariana*.
R. americana (Nutt.) Kuntze on *Pinus contorta*.
R. douglasii (Engelm.) Kuntze on *Pseudotsuga taxifolia*.
R. douglasii, var. *abietina* Engelm., on *Abies grandis* and *A. lasiocarpa*.
R. douglasii, var. *microcarpa* Engelm., on *Picea engelmanni*.

A glance at the foregoing list shows a very interesting association of mistletoes. The form on *A. lasiocarpa* (Pl. LV, fig. 3), as known to the writer, in point of morphology, color, and the time of maturity of pollen and seed, coincides with the form on *A. grandis* (Pl. LV, fig. 2). The mistletoe on *Picea engelmanni* is slightly smaller, often very much so (Pl. LV, fig. 4), but its other characteristics are the same. Comparing these mistletoes with *R. douglasii* and *R. pusilla*, there is at once a marked similarity among all five. They do not vary widely in form and color of the stems. There is some variation in point of distribution of the individual plants on the branch, whether aggregated or appearing singly. Any one mistletoe, however, may exhibit both or either condition. The staminate flowers of all five are a deep rich purple. No other species of the genus possesses this character to such a marked degree. All five bloom at the same time in the same latitude and exposure, and the seeds ripen and are expelled in the same month.

The question naturally arises, What is the true taxonomic position of these mistletoes? Engelmann recognized the close affinities of the small forms on spruce and fir to *R. douglasii* and named them varieties of that species. The isolated and infrequent occurrence of these small mistletoes on spruce and fir in the West should throw some light on their probable relationships. If they are specifically distinct, they should show greater activity in attacking their hosts. As it is, a single tree will bear a few plants (broom formation) and the most diligent search on the same host for miles around will not reveal a second infection. The discovery of *W. arceuthobii* on those forms or species of the same

genus in the West which are most similar to the eastern-spruce mistletoe may have some bearing on the taxonomic position of this group of mistletoes. The occurrence of the fungus on *R. americana*, a very definitely associated and characteristic species with no affinity whatever with the mistletoes of the Pseudotsuga-Abies-Picea group, indicates a cosmopolitan character for the disease. To determine this point, the fungus has been introduced into clumps of the yellow-pine and larch mistletoes. These experiments are now under way.

MORPHOLOGY

Photographs of *W. arceuthobii* have not been published. For this reason detail enlargements from the original negatives of infected and uninfected fruits of *R. americana* are reproduced in Plate LVI, figures 1 and 2. Reproductions of photographs of the fungus (natural size) on all its western hosts, so far as known, are likewise shown (Pl. LV, fig. 1–5). These illustrations indicate very clearly the interesting habitat of the fungus. A study of the enlargements (Pl. LVI, fig. 2) shows the shiny black perithecia densely crowded at the apex of the fruit. Varying numbers, sometimes as many as 40 or more, have been counted springing from the brownish black stroma within the seed capsule. The general shape of the perithecia is that of an oblong cylinder. Usually, however, they are slightly enlarged at the free ends and very abruptly rounded. The hyphæ composing that part of the stroma from which the perithecia take their origin are densely compacted, brown or black, with thick walls. Deeper within the capsule, the brown color is not so conspicuous, although the mycelium is generally brownish. The outer walls of the perithecia are uniformly smooth; very rarely a 4- to 6-celled projection is present. The crowded condition of the perithecia often gives them the appearance of being partially embedded in the stroma. The wall between two perithecia when densely crowded may be very thin and appears to be occasionally ruptured on the escape of the spores from the asci. The asci show considerable variation in shape, owing principally to their crowded condition, but when free are uniformly pear- or club-shaped, with fairly long pedicels. Probably in no other species of the order is the early disappearance of the wall of the ascus so characteristic. Before the spores have reached maturity or at least before they have assumed the normal color of mature spores, the ascus wall disappears. The ascus is probably ruptured at the apex by the pressure of the developing spores within. That a considerable pressure must be exerted against the walls of the ascus is shown by the fact that the spores when free are normally spherical, but within the ascus they are often bluntly angular. They often persist in clumps after their escape from the ascus. The asci vary but very little in size. The measurements (Zeiss filar micrometer with No. 12 compensating ocular

and 8 mm. n. a. 0.65 apochromatic objective) show a close uniformity to those of the type material. The measurements of the asci from fresh material range as follows: 22.3, 22.8, 24, 24.4, 24.8, 25.2μ in length. Evidently considerable shrinkage takes place in stained material, the stained asci measuring 16.5, 16.9, 19.8, 21.9μ in length. The average breadth of the ascus is 3μ. The ascus contains eight unicellular, globose, thick-walled spores. The spores are at first hyaline, but nearing maturity they assume a very conspicuous brown-black color. The color of the mature spores is assumed after their escape from the ascus. The preliminary color changes may, however, take place within the ascus. Prof. Peck, in his original description, states that the spores are hyaline; still he represents, in his illustration (Pl. LV, fig. 14), four mature spores which are black. The change from a hyaline to the pronounced brown or black color was evidently recognized, since "an ascus containing young spores" is represented, after which "four mature spores" that are black are represented. An examination of some of the type material kindly sent the writer by Mr. H. D. House shows the spores in all stages of development and varying from hyaline to black. The dimensions of the spores in the type material are found to agree with the measurements of the spores in the western fungus, which range as follows: Unstained and out of ascus, 3.7, 4.5, 4.9, 5.3, 5.8, 6.2μ; stained, 4.1, 4.5, 4.9, 5.3, 6.2μ. Previous accounts give the diameter of the spores as "about 4μ." The paraphyses are filamentous, short, and very inconspicuous.

All the asci of a single perithecium do not mature their spores together; instead, at the time mature spores are escaping from the perithecium, young asci showing early stages of spore differentiation are discernible. There is consequently a gradual dissemination of the spores, governed to an extent by the humidity of the atmosphere. The opening through which the spores escape is directly at the apex and is formed by the free ends of the thick-walled hypha composing the walls of the perithecium. The cells composing the tips of these hyphæ seem to possess certain hygroscopic properties, as they are observed to bend in or out on the addition or absence of moisture.

BIOLOGY

In what manner the spores of *W. arceuthobii* are conveyed to the pistillate flower of the mistletoe in nature the writer is not in a position to state definitely. Since isolated infections occur promiscously on different branches of the same tree or on different trees in the same locality, it is evident that the wind is the chief factor in spore dissemination. A fact observed among the infected plants on lodgepole pine of the swamp area previously mentioned supports this view. The area lay with its long axis in the direction of the prevailing winds of the Priest River Valley. An examination of the trees bearing infected plants showed that they were more or less in line with each other and extended

in the direction of the most constant winds. On either side of the most heavily infected area the trees did not support infected plants, although the mistletoe was abundant. Furthermore, large compact brooms always bore the greater number of infected plants on the windward side.

The writer has recently determined that insects to a certainty play a rôle in the pollination of these mistletoes. Hymenopterous insects are chiefly in evidence, but those of other orders are also known to promote pollination. During 1914 grasshoppers in great numbers came out of the Hangman Creek Valley near Spokane and fed upon blooming staminate plants of the large mistletoe growing in profusion on yellow pine of the bench lands. These insects seemed to select only the flowers of the staminate plants for food; but, swarming over the pistillate plants, they deposited some of the pollen that adhered to their bodies. It is as easily possible that the spores of the mistletoe fungus are in a minor degree transported in a like manner. Rain dropping from infected to uninfected plants or running down the pendent branches and dropping off at the tips of the mistletoe plants is probably a factor in distributing the disease on any one tree or broom. It so happened at the field station that a number of newly collected infected capsules were left overnight and a portion of the following day on a glass slide under the microscope. An examination of the slide showed that a number of spores had been expelled and lay in a ring about $\frac{1}{2}$ mm. away from the apex of the perithecium. Evidently there is a slight expulsion of the spores under favorable conditions. This came as a surprise, as the stiff ends of the hyphæ forming the perithecial wall seem to open with difficulty. A number of perithecia collected from fallen capsules in the spring still contained numerous spores. The early disappearance of the ascus within the perithecium precludes any expulsion from this source. The force must arise from the continual maturing and crowding of the spores toward the outward end of the perithecium. Under favorable conditions this pressure may become sufficient to force the spores out through the aperture. It has already been indicated that a pressure seems to exist within the perithecium. This force, though weak, may still be sufficient to cause the spores to land on capsules of the same plant that escaped previous infection.

The spores of the fungus are beginning to ripen and to be expelled from the perithecia in the latitude of northern Idaho about the end of November and are capable of germinating immediately. The method of penetration of the germ tube of the spore into the developing fruit of its host has not as yet been observed. Since a considerable period elapses between pollination and the time actual fertilization takes place in the host, it is quite possible that the germination of the fungus spore coincides with the advance of the pollen tube toward the embryo sac. This would enable the germ tube of the spore to travel toward the ovule of its host by a line of least resistance. In early spring, or at the time

actual fertilization of the mistletoe takes place, those tissues destined to become the seed are, in infected plants, observed to be completely filled or destroyed by the mycelium of the fungus. After infection, the young seed capsule never increases much in size and is entirely dominated by the parasite. The diseased capsules usually fall away during late winter and early spring, which allows time for the infection of the pistillate plants. The drain on the vigor of the mistletoe plant, if all the young capsules are infected, is such that it may also succumb and fall. If only one or two capsules of the plant are infected, it will remain intact, maturing the uninfected fruit of the season and fruiting again the following year. Usually, however, the infection of all the fruits of a mistletoe colony or of all the plants of a broom is so complete that few or no seeds mature.

ECOLOGY

All collections so far made of the fungus have not been at an elevation much greater than 3,600 feet, although its hosts may range well up toward the timber line. This indicates a preference for the conditions of the lower levels, where it is not so much exposed to fluctuations of warmth and moisture. The latter factor is probably of greater influence. Until the fungus is found elsewhere it may be said to prefer the North Temperate regions. Forestburg, N. Y., its first known station, is about on a line with the Upper Peninsula of Michigan, the region of its second discovery, and northern Idaho, where it was last found. This is its geographical and climatic range at present. Developing either on exposed or shaded plants, the fungus seems to favor those growing in shaded positions, such as the inner parts of brooms. Absence of direct sunlight may promote development, but, after the capsule becomes infected, direct sunlight can not have much influence on the maturing of the fungus. The germination of the spores would probably be promoted by an absence of direct sunlight. Warm fall rains, such as occur in northern Idaho, are undoubtedly very favorable to the development and spread of the disease, since in this region the fungus has been found most abundant. In damp river bottoms or on the borders of swamp areas the lodgepole-pine mistletoe, which frequently occurs in profusion in such a habitat, is very likely to be attacked by the fungus. Prof. Peck [1] does not record the conditions under which the fungus was growing at Forestburg, N. Y., but presumably it was a region of considerable humidity. The Upper Peninsula of Michigan, where Prof. Wheeler collected the fungus, is a region of numerous swamps and abundant atmospheric moisture. In view of the fact that the fungus is parasitic on the rather succulent capsule of the mistletoe, atmospheric humidity should not greatly interfere with its life functions, except probably in the initial stages of spore germination. The fungus should thrive on the larch

[1] Peck, C. H. Op. cit.

mistletoe, provided it is susceptible to attack, owing to the usually damp condition of the compact moss-covered brooms. It remains to be seen under just what conditions the fungus will propagate itself. To this end it is being introduced into mistletoe regions of all types of exposure.

The ease with which the fungus seems to infect its host leads the writer to believe that it may be of some economic importance in the control of certain species of mistletoe, at least for small areas. For a mistletoe species to propagate itself, it must produce seeds abundantly, in order to insure the infection of the young growing forest. The proportion of mistletoe seeds actually causing infection to the total number produced is very small indeed. Some fall to the ground; some fall on plants not susceptible; most of them fall on parts of the host too old to be penetrated by the young root of the seed. With the exception of a few rare instances, where infections have been known to occur on wound tissue of mature parts of trees, the writer has not yet found either in nature or by actual inoculation a seed taking effect on any part of its host other than the more tender shoots or their equivalents in tenderness of bark and then only when the primary sinker found its way to a leaf scar, leaf scale, or other more vulnerable irregularities of the substratum. Again, the seed must fall in such a position that the protruding root may directly find its way under a leaf scale or be sheltered by the thick bunch of needles at each node of growth or at the base of a leaf or leaf sheath; otherwise it may fail of its purpose. The seed may germinate and expend its stored materials in the production of a primary root of half an inch or more, but before the growing point can penetrate the stem, provided it is in such a position as to be drawn toward it, the young hypocotyl is exhausted. Very few seeds cause an infection when not very favorably located or directly through the smooth epidermis possessing a suberized layer.

With the exception of the small forms mentioned in this paper most of the members of the genus are prolific seed producers. If so few seeds find a vulnerable point on their hosts even with an abundant production of seed, so much less will the chances of infection be if the seed production is lessened. An estimate of the number of seed that should have been produced by the lodgepole-pine mistletoe on a small broom was about 400. Not a single mistletoe seed on this broom had reached maturity. All were attacked by the fungus. The biologic control of organic agents destructive to plant life is in most cases a thing very much in the realm of fancy. It seems, however, that a fungus of the nature of *W. arceuthobii* may be introduced into mistletoe regions possessing certain climatic conditions with the prospect of reducing the seed production of these parasites, and thus reducing the damage caused by the mistletoe.

SUMMARY

Wallrothiella arceuthobii, a fungous parasite on the false mistletoes of conifers, is reported for the first time in the West.

This fungus, first collected by Prof. Peck in New York and again by Prof. Wheeler in the Upper Peninsula of Michigan, was considered a very rare species until it was found to be of common occurrence in parts of Montana and Idaho.

Several new facts pertaining to the morphology and general behavior of the fungus are established.

Its host range has been greatly extended.

The significant fact that the fungus is found in the West on those forms of species of the same genus which are most similar to the eastern black-spruce mistletoe, its host in the East, is thought to have some bearing on the taxonomic position of this particular group of mistletoes.

Its parasitism on the false mistletoes is found to be of great significance in the control of these parasites, which are so destructive to many western conifers.

Fig. 1.—*Razoumofskya douglasii* on *Pseudotsuga taxifolia*, infected with *Wallrothiella arceuthobii*. Note that two capsules escaped infection. Natural size.

Fig. 2.—*R. douglasii*, var. *abietina*, on *Abies grandis*, infected with *W. arceuthobii*. Natural size.

Fig. 3.—*R. douglasii*, var. *abietina*, on *Abies lasiocarpa*, infected with *W. arceuthobii*. Natural size.

Fig. 4.—*R. douglasii*, var. *microcarpa*, on *Picea engelmanni*, infected with *W. arceuthobii*. Natural size.

Fig. 5.—Left and right figures showing infection of *R. americana* with *W. arceuthobii* by infected plants of *R. douglasii*. The plants at lower part of figures are normal and fully mature. The middle figure shows infection of *R. americana* by spraying upon the plants a mixture containing spores of *W. arceuthobii*. Natural size.
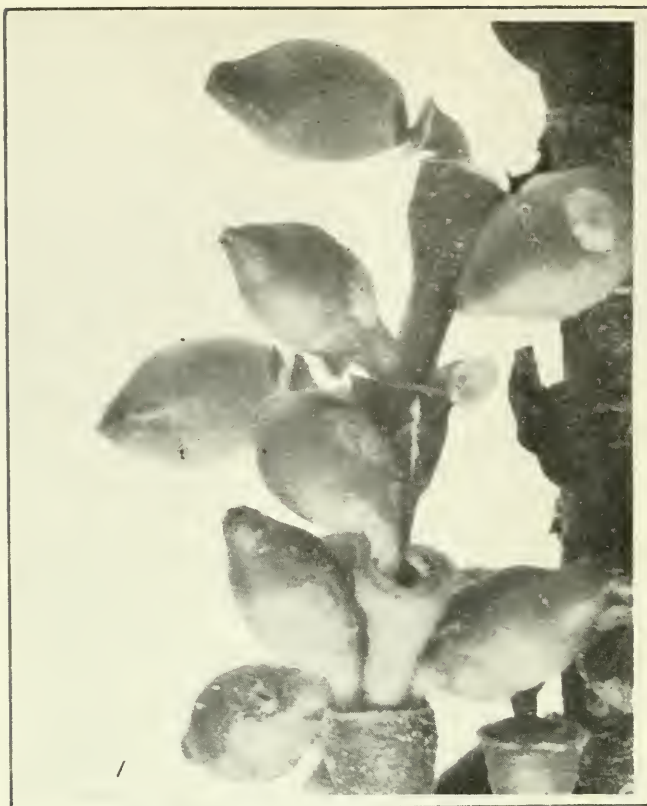
TENSILE STRENGTH AND ELASTICITY OF WOOL

By Robert F. Miller, *Assistant in Animal Husbandry, Montana Agricultural Experiment Station*, and William D. Tallman, *Professor of Mathematics, Montana State College of Agriculture*

INTRODUCTION

The study of the tensile strength and elasticity of wool is comparatively a new line of investigation. McMurtrie,[1] of Illinois, in cooperation with the United States Department of Agriculture, tested a great number of fibers, and Prof. J. A. Hill, of the Wyoming Agricultural Experiment Station, Laramie, Wyo., did considerable work along this line. Other than that, so far as the writer knows, there has been no research on this problem in the United States.

The exact structure of wool is not well understood. It differs from a hair in that the medullary, or central, layer of cells, corresponding to the heart of a tree, is absent in true wool. The coarse wools, however, possess this layer to some extent and, hence, might be termed "hair" in one sense of the word. The line of distinction can not be sharply drawn.

McMurtrie [1] measured the breaking strain and elasticity of over 35,000 fibers, representing nearly 1,000 samples. He tested 30 fibers per sample to secure a fair average test. In another series tested a few years later a small lock was taken from each sample and the fibers were drawn at random. In this way it was found necessary to test 50 fibers of each sample to obtain a true average.[2]

Matthews [3] makes the following statement in regard to this point:

A fair average of breaking strain and elasticity may be obtained for any quality of fibre by testing about 10 separate fibres and taking the mean of the total tests. If the quality of fibres, however, in a sample does not run very uniform it is best to increase the number of tests to 25 or even 50 in order that a satisfactory average may be obtained.

Prof. Hill and assistants tested in all 59,400 fibers and proved the fallacy of using only 10 to 50 fibers for a true average. He tested 1,000 fibers per sample and divided them into groups of 100. Four of the means of the groups of 100 differed from the mean of the 1,000 by more

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[1] McMurtrie, William. Report upon an Examination of Wools and Other Animal Fibers ... made under the direction of the Commissioner of Agriculture. p. 217. Washington, D. C., 1886. Pub. by the U. S. Dept. Agr.

[2] Id., p. 425.

[3] Matthews, J. M. The Textile Fibres ... ed. 1, p. 274. New York, 1904.

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(379)

than 4 per cent, and between the mean of the sixth and the mean of the seventh hundred there was a difference of 14.63 per cent. He says: [1]

A result such as this from the first experiment shows that, even if this were an exceptional case, it is at least necessary to test several more samples before deciding to base any conclusions upon means of so few as 100 measurements.

At the Wyoming Experiment Station as high as 10,000 fibers from one sheep were tested for breaking strain and divided into groups of 5,000 each, on which Hill comments as follows: [2]

Notwithstanding the fact that for most purposes the drawing and testing of 5,000 fibers of wool in order to determine the tensile strength of a sample would be highly impracticable, Table III shows that where the samples were not carefully mixed before drawing the sub-samples, the means of this number in two samples differ by more than 5% when only two and three means are compared.

NECESSITY FOR A METHOD OF TESTING WOOL

On July 1, 1908, a project in wool research was undertaken by Prof. R. W. Clark, of the Montana Experiment Station. The object was to study the effect of various factors—feeding, breeding, care, management, etc.—upon the wool and form of the sheep.

The first step necessary was to work out a method to test accurately the qualities of the wool fibers. This has proved a long and tedious task, owing to the excessive variation among the fibers.

In the sample of Rambouillet wool, fibers grown side by side varied in breaking stress from 20 to 140 dgm. Also the stretch or strain varied from 1 to 15 mm. Hence, it is self-evident that a great many fibers would have to be tested to reduce this variation to a minimum.

Table I gives the results obtained by testing 1,000 fibers from the same place on the body of the sheep for breaking stress. Five samples were taken from each sheep.

TABLE I.—*Results of testing fibers of wool for breaking stress*

Sheep No.	Shoulder.	Back.	Side.	Belly.	Hip.
	Dgm.	*Dgm.*	*Dgm.*	*Dgm.*	*Dgm.*
324	55.994±0.404	53.127±0.407	56.566±0.502	58.13 ±0.437	54.652±0.496
6283	56.146±0.452	53.894±0.502	50.523±0.440	51.868±0.298	62.702±0.575
6382	55.717±0.325	57.56 ±0.431	63.502±0.417	46.97 ±0.298	65.696±0.576

To explain the notation, let us consider for an illustration the shoulder of sheep No. 324, which is given as 55.994±0.404. By this is meant that the average breaking stress of the 1,000 fibers was 55.994 dgm., and the probable variation for the 1,000 was 0.404 dgm.—i. e., the chance is even that if another lot of 1,000 fibers were taken from the same place, the

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[1] Hill, J. A. Studies on strength and elasticity of the wool fiber. 1. The probable error of the mean. Wyo. Agr. Exp. Sta. 21st Ann. Rept., 1910/11, Sup., p. 16. 1911.
[2] Hill, J. A. The value of fiber-testing machines for measuring the strength and elasticity of wool. Wyo. Agr. Exp. Sta. Bul. 92, p. 22. 1912.

average breaking stress would lie between $55.994 - 0.404 = 55.590$ dgm. and $55.994 + 0.404 = 56.398$ dgm. In this case the probable variation of a single fiber would be 12.764—i. e., we should expect that one-half of the fibers would have a breaking stress lying somewhere between 43.230 and 68.758 dgm.

At the same time that these observations were taken, a record was kept of the ultimate strain of each fiber. But no account was taken of the length of each fiber at the time stress was imposed. However, it was evident that the ultimate strain was very slightly, if at all, a function of the breaking stress.

BREAKING STRESS AND TENSILE STRENGTH[1]

The first work on this project was the determining of the breaking stress of the fibers and the probable variation in the same, for the purpose of getting an average with a probable variation so small that it could be disregarded. It was found, however, that the variation was too great to give conclusive results even when using as high as 5,000 fibers per fleece. This was to be expected in part, as to compare wool fibers without regard to their sizes does not seem to be practicable.

It was decided, therefore, that the ultimate breaking stress of the fibers was not what was wanted, but rather the quality of the fabric woven from the wool. The strength of the fabric depends upon the tensile strength of the fibers—i. e., the breaking stress divided by the cross section of the fiber.

Each fiber of coarse wool has a greater breaking stress than single fibers of fine wool, yet a piece of goods made from the fine wool will contain many more fibers to the yard than one made from the coarse wool; thus, the former may make the stronger cloth. It is therefore evident that we must know the cross section of each fiber as well as the breaking stress. The results from testing the fibers on this basis is illustrated by the following samples of wool: Rambouillet No. 6401—breaking stress, 49.35 dgm.; tensile strength, 100.988. Shropshire No. 67—breaking stress, 140.00 dgm.; tensile strength, 119.886. It will be seen that while the ratio of the breaking stress is 1 to 2.83, that of the tensile strength

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[1] EXPLANATION OF TERMS USED:

Length (L)=length of fiber tested, in millimeters.

Diameter (D)=measured on the microscope with a micrometer eyepiece. 1 unit equal 1.5μ.

Area (A)=area of cross section of fiber. Formula $\frac{\pi D^2}{4}$.

Breaking stress (S)=force required to break fiber, in decigrams.

Strain (E)=stretch or elongation of fiber, in millimeters.

Tensile strength (TS)=strength per unit area $\frac{S}{A}$. However, to save time in our calculation we computed a value $\frac{S}{D^2}$ which is the constant $\frac{4}{\pi} \times TS$.

Elastic limit (EL)=point at which if more force is applied, Hook's law $\frac{S}{E} = K$ breaks down.

Young's modulus (Y)=a formula used for measuring material which is based on Hook's law. $\frac{\text{Stress}}{\text{Strain}}$=a constant. Formula, $\frac{L \times S}{E \times A}$.

Probable variation (PV)=a value which in the long run is greater than the variation of exactly one-half the fibers from the mean.

is only 1 to 1.19. Thus, in comparing the breaking stresses we get a greatly distorted idea of the difference in the strength of the fabric that would be manufactured from the wool.

A large number of tests were then made to determine the tensile strength of the fibers. The results are given in Table II.

TABLE II.—*Results of testing fibers of wool for tensile strength—sheep 6398; sample from hip*

Lot of fibers.	Stress.	Coefficient of variation.	Tensile strength.	Coefficient of variation.
	Dgm.	*Per cent.*	*Dgm.*	*Per cent.*
First 100......................	46. 28±1. 53	3. 31	74,765±2,146	2. 65
Second 100....................	44. 75±1. 26	2. 82	75,293±1,573	2. 09
Third 100.....................	51. 73±1. 19	2. 30	76,973±1,452	1. 88

This shows an average coefficient of variation of 2.81 per cent for the stress and 2.21 per cent for the tensile strength.

ELASTIC LIMIT

However, there are other elements that are even more important than the tensile strength. We do not care so much that a fabric shall have a high resistance to a tearing force as that it shall have wearing qualities. If we catch our clothes on a nail and tear them, the force exerted is usually so great that any slight variation in the strength of the cloth will make no difference. Young's modulus, which is the stress

FIG. 1.—Curve showing the regularity of stretch and the abrupt break of merino wool after the elastic limit is passed. X=elastic limit.

per unit area of cross section divided by the strain per unit length, will show the degree with which a fabric will withstand deformation under ordinary forces. Thus, we expect to find that when the wool has a high

Young's modulus a fabric manufactured from it will have high resistance to deformation of shape.

By what is known as Hook's law, the stress divided by the strain is constant to a certain point and from that time on the ratio decreases. This point is known as the elastic limit of the substance, and in determining Young's modulus we always take the observations before the elastic limit is reached. When a force is applied that carries the deformation beyond the elastic limit, the wool will not come back to its original shape. In fact, this force has begun to tear apart the molecules of the fiber, and the wool has become permanently weakened. Thus, a record of the elastic limit for the fibers is essential.
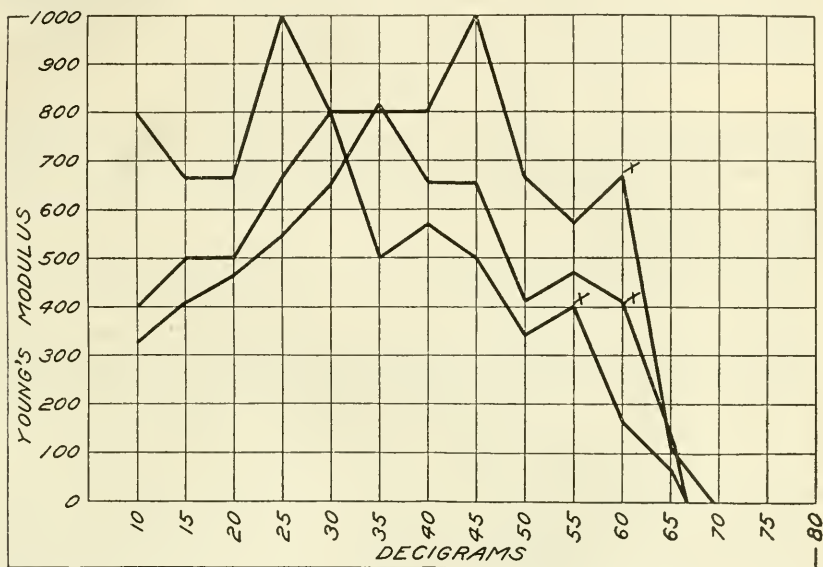
Fig. 2.—Curve showing Young's modulus of elasticity of merino wool at different stresses. X=elastic limit.

Figures 1 and 2 show the curves illustrating the elastic limit. The former shows the stretch or strain as force is applied; the latter shows the modulus curve.

Table III gives the variation in the elastic limits for various fibers, as obtained from three sheep.

TABLE III.—*Variation in the elastic limits of wool fibers of three sheep*

Sheep No.	Column 2.	Column 3.
	Dgm.	*Dgm.*
81	42. 90±0. 7608	17. 54±0. 2320
82	34. 95± . 8768	17. 54± . 2934
676	79. 90±1. 7476	17. 46± . 2901

In column 2 is given the average tension per wool fiber at the time of passing the elastic limit. In column 3 is given the average tension at the time of passing the elastic limit on an area of the wool fiber equivalent to the area of cross section of a wool fiber whose diameter is 15μ. The first two of these sheep were range-bred merinos and the third was a pure-bred Shropshire. The Shropshire has much coarser wool fibers; consequently the large value in column 2. But, in spite of the difference in breed and a large variation in the size of wool fibers between Shropshires and merinos when reduced to the tension on equal areas of cross section, we find great uniformity for the three sheep. These three sheep had received similar care and feed. We should expect a greater variation in elastic limit than shown above for sheep of the same breed under different conditions as to feed, shelter, etc.

DESCRIPTION OF TESTING APPARATUS

The apparatus used in these tests consists of (1) a fiber-testing machine devised for the Philadelphia Textile School and (2) a compound microscope with micrometer eyepiece attached for accurate measuring of the diameter of the fibers.

The following description of the fiber-testing machine is given by Matthews [1] (Pl. LVII, fig. 1 and 2).

The fibre to be tested is clamped between the jaws at (J), the pointer attached to the end of the beam above the upper jaw being brought to the zero-mark on the scale (S), while the lower jaw is raised or lowered in its stand until the desired distance between the jaws is obtained. To obtain comparable results this distance should always be the same. [We have used 40 mm. for our observations.] The sliding-bar (R) is moved forward by turning the rod (T), which moves the rack and pinion at (P), until the graduation on the wheel (G) is at zero to the indicator. Under these conditions there is no strain on the fibre. A stretching force is then placed on the fibre by moving the bar (R) backward by turning the rod (T); the motion of this bar is made uniform and gradual until the fibre finally breaks under the strain thus placed upon it. The graduation on the wheel (G) will then indicate in decigrams the breaking strain of the fibre being tested. The elasticity is obtained by watching carefully the pointer moving up the scale of millimeters at (S) until the rupture of the fibre takes place; the distance this pointer moves represents the actual stretch of the fibre. . . . The weight (W) at the rear end of the beam can be moved backward or forward, and is for the purpose of adjusting the balance so that there is no strain at (J) when the indicator (G) marks zero. The wheel (G) is graduated in decigrams, and this marks the sensibility of the machine; the total graduations on (G) running from zero to 400. When fibres are tested having a greater tensile strength than 400 decigrams a fixed additional weight of 10, 25, 50, etc., grams may be hung from (W), and this must be added to the reading on the wheel when the fibre breaks. If the elasticity of the fibre is so great as to carry the pointer beyond the limits of the scale at (S), a shorter length of fibre must be tested.

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[1] Matthews, J. M. Op. cit., p. 272–274, fig. 69.

IMPROVED TESTING APPARATUS

Because of the difficulty of making accurate readings on the scale of the fiber-testing machine, the following apparatus was devised to be used with it:

(1) An illuminated scale with the lamps inclosed to prevent reflection; (2) an optical lever attached to the testing machine; (3) a large plain mirror; and (4) a high-power telescope.

The instruments are so arranged that the illuminated scale, A, is thrown onto the optical lever, B, by means of which it is reflected onto the mirror, C, from which it is read through the telescope, D (Pl. LVII, fig. 3).

The telescope magnifies about 28 times, and the distance between the scale and the optical lever is such that the total magnification is just 50. In this way very accurate readings can be taken.

To test a fiber, the machine is first balanced so that it is in perfect adjustment with the optical lever attached. A fiber is then put between the jaws of the machine and 10 dgm. of force applied to take out the crimp or waviness, making the fiber perfectly tight. A reading is taken at this point and again when 15 dgm. of force is applied and again at every 5 dgm. additional until the fiber passes the elastic limit. After the elastic limit is passed, force is gradually applied until the fiber breaks. A portion of it is mounted on a slide and the diameter obtained under the microscope by means of a micrometer eyepiece. Young's modulus and tensile strength can then be determined.

EXPLANATION OF DIAGRAMS

Mention has been made of the great variation in the fibers taken from the same place upon the sheep's body. By means of figure 3 we can express these variations more definitely. If, for example, we are investigating the tensile strength of fibers, the best value to take would be the average of that found for the separate fibers. But, if observations had been taken on a hundred fibers, we should not expect that average to be the same as for another hundred taken from the same place. It is therefore necessary not only to take the average but the probable variation— i. e., an amount such that, if added to and subtracted from, the average obtained will give two numbers such that the average for another hundred would have even chances of lying between these two numbers. If two samples of wool from different places on a sheep, from different sheep, or from the same place under different treatment have been examined and the tensile strength with probable variation determined, we must have a means of knowing with what certainty we may state that one is stronger than the other and by how much. While the fibers which have the highest average will have a probability of being the strongest, that probability may be very slight and, in fact, so slight that we hardly dare make any
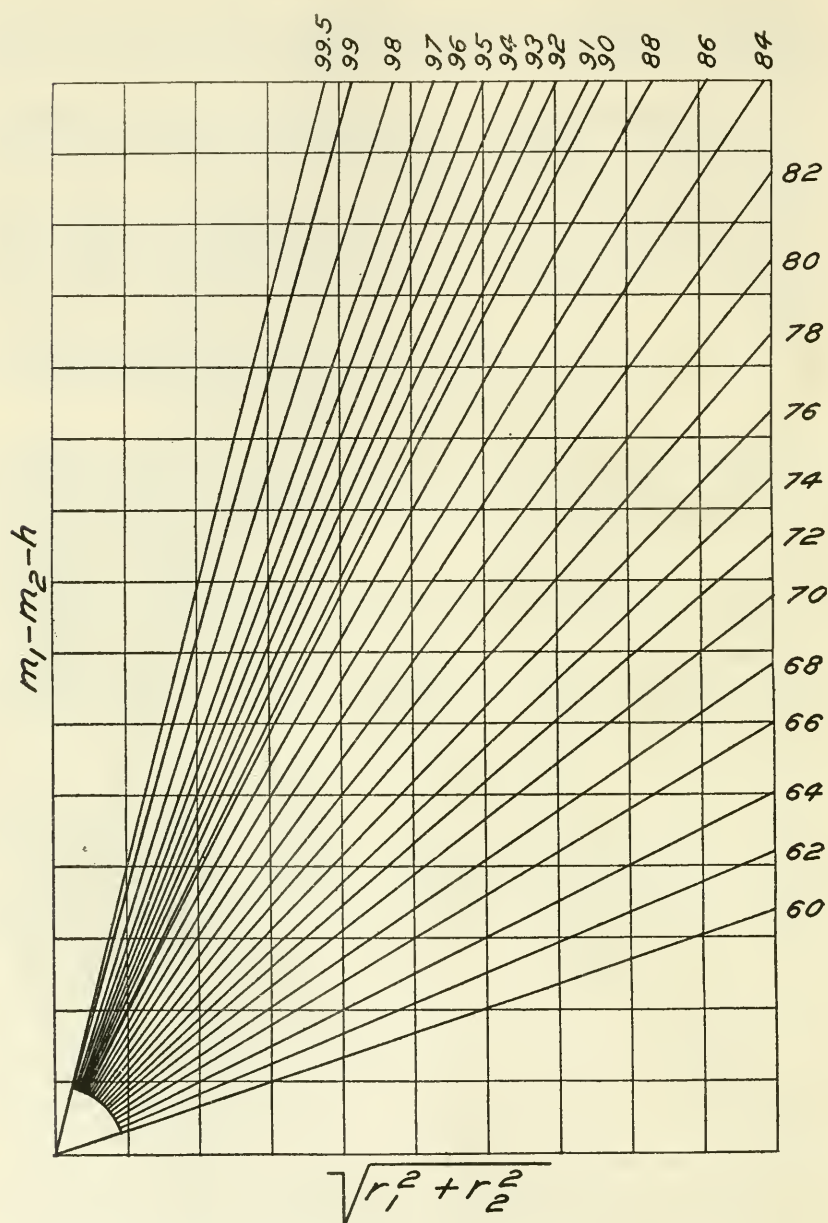
FIG. 3.—Diagram for plotting curves by means of which the percentage of accuracy of the tensile strength of wool when comparing two sets of observations may be exactly calculated.

statement as to their relative strength.   To give definite information as to their relative strength, when the averages and probable variations are given, figure 3 has been devised, from which may be read the percentage probability that one lot of fibers is stronger than the other; also, the percentage probability that it is stronger by any given amount.

FIG. 4.—Curve of ordinary probability. The abscissæ are taken as a variation in strength of a given fiber from the mean strength of all the fibers and the ordinates are proportional to the probability that such a fiber exists.

Suppose two sets of observations give $m_1 \pm r_1$ and $m_2 \pm r_2$ and we wish to show the probability that the first is greater than the second by an amount h. Knowing the probable variation, r, a curve can be drawn whose highest ordinate is to the ordinate of any point whose abscissa is x as the number of fibers whose strength is the average is to the number of fibers whose strength is x more than the average.   In this case, we take x to be either a positive or a negative number.

The equation of this curve is $y = \dfrac{.4769}{r_1\sqrt{\pi}} e^{-\left[\frac{.4769x}{r_1}\right]^2}$.   Figure 4 is an illustration of this curve.

The probability that the actual strength of fibers recorded as $m_2 + r_2$ is within dx of $m_2 + x$ is $\dfrac{.4769}{r_2\sqrt{\pi}} e^{-\left[\frac{.4769x}{r_2}\right]^2}$.

The probability that the strength of fibers given as $m_1 \pm r_1$ is $m_2 + x + h$ or just h greater than the other is

$$\frac{.4769}{r_1\sqrt{\pi}} e^{-\left[\frac{.4769(m_2 - m_1 + x + h)}{r_1}\right]^2} dh.$$

Hence, the probability that the fibers given as $m_2 + r_2$ have a real strength $m_2 + x$ and the other just h greater is

$$\frac{(.4769)^2}{r_1 r_2 \pi} e^{-\left[\frac{.4769x}{r_2}\right]^2} e^{-\left[\frac{.4769(m_2 - m_1 + x + h)}{r_1}\right]^2} dx . dh.$$

And the probability that the former has a strength $m_2 + x$ and the latter a strength at least h greater is

$$\frac{(.4769)^2}{r_1 r_2 \pi} dx \int_h^\infty e^{-\left[\frac{.4769x}{r_2}\right]^2} e^{-\left[\frac{.4769(m_2 - m_1 + x + h)}{r_1}\right]^2} dh.$$

Now, if we let x take all possible values, we have as the probability that the group recorded with the strength $m_1 + r_1$ is at least stronger than the other the double integral

$$\frac{(.4769)^2}{r_1 r_2 \pi} \int_{-\infty}^{\infty} \int_h^{\infty} e^{-\left[\frac{.4769x}{r_2}\right]^2} e^{-\left[\frac{.4769(m_2 - m_1 + x + h)}{r_1}\right]^2} dx.dh.$$

Designating this probability by p and letting $\frac{.4769}{r_1}(m_2 - m_1 + x + h) = t$, we shall have the equation

$$p = \frac{.4769}{r_2 \pi} \int_{-\infty}^{\infty} \int_{\frac{.4769}{r_1}(m_2 - m_1 + x + h)}^{\infty} e^{-\left[\frac{.4769x}{r_2}\right]^2} e^{-t^2} dx.dt.$$

By breaking this integral into two, we may write our equation as follows:

$$p = \frac{.4769}{r_2 \pi} \int_{-\infty}^{\infty} \int_0^{\infty} e^{-\left[\frac{.4769x}{r_2}\right]^2} e^{-t^2} dx.dt.$$

$$- \frac{.4769}{r_2 \pi} \int_{-\infty}^{\infty} \int_0^{+\frac{.4769}{r_1}(m_2 - m_1 + x + h)} e^{-\left[\frac{.4769x}{r_2}\right]^2} e^{-t^2} dx.dt.$$

The first of these integrals can be evaluated by a well-known method in definite integrals. Its value is $\frac{1}{2}$. The second integral we shall call q. Then placing $m_2 - m_1 + h = k$, we obtain the following:

$$q = \frac{.4769}{r_2 \pi} \int_{-\infty}^{\infty} \int_0^{+\frac{.4769}{r_1}(k + x)} e^{-\left[\frac{.4769x}{r_2}\right]^2} e^{-t^2} dx.dt$$

$$\frac{dq}{dk} = \frac{(.4769)^2}{r_1 r_2 \pi} \int_{-\infty}^{\infty} e^{-(.4769)^2 \left[\left(\frac{x}{r_2}\right)^2 + \left(\frac{k + x}{r_1}\right)^2\right]} dx.$$

or

$$\frac{dq}{dk} = \frac{(.4769)^2}{r_1 r_2 \pi} e^{-\frac{(.4769k)^2}{r_1^2 + r_2^2}} \int_{-\infty}^{\infty} e^{-\left[.4769\left(\frac{x\sqrt{r_1^2 + r_2^2}}{r_1 r_2} + \frac{k r_2}{r\sqrt{r_1^2 + r_2^2}}\right)\right]^2} dx.$$

Now, by putting $.4769\left(\frac{x\sqrt{r_1^2 + r_2^2}}{r_1 r_2} + \frac{k r_2}{r_1\sqrt{r_1^2 + r_2^2}}\right) = u$ we shall have

$$\frac{dq}{dk} = \frac{.4769}{\pi\sqrt{r_1^2 + r_2^2}} \int_{-\infty}^{\infty} e^{-\frac{(.4769k)^2}{(r_1^2 + r_2^2)}} e^{-u^2} du.$$

Integrating with respect to u, we have $\dfrac{dq}{dk} = -\dfrac{.4769}{\sqrt{\pi}\sqrt{r_1^2 + r_2^2}} e^{-\frac{(.4769k)^2}{r_1^2 + r_2^2}}$.   Substi-

tuting $\dfrac{.4769k}{\sqrt{r_1^2 + r_2^2}} = s$ and putting the equation into the integral form,

we shall have $q = \dfrac{1}{\pi} \displaystyle\int_0^{\frac{.4769k}{\sqrt{r_1^2 + r_2^2}}} e^{-u^2} du$.   Now, tables are made for the integral

$\displaystyle\int_0^\infty e^{-t_2} dt$ when t is a constant.   Now, $k = m_2 - m_1 + h$; r_1 and r_2 are known,

so q can be taken from this table.   Returning to p, we have $p = \dfrac{1}{2} - q$.
The value of q evidently depends upon the ratio of $-k$ to $\sqrt{r_1^2 + r_2^2}$.
Taking these two values as the ordinate and the abscissa of a point, it is
evident that all points on a straight line passing through the origin of
coordinates would give the same value p.   Given p, we may determine
the slope of the line corresponding to the given value of p.   On a sheet
of cross-section paper (fig. 3) lines have been drawn corresponding to
certain values of p.

As an example of the use of this diagram, observations on two sets of
wool gave as their tensile strengths the following results: (*a*) 101,180±.
2,219 and (*b*) 75,293±1,573 dgm. per sq. mm. $m_1 - m_2 = 25,887$
$\sqrt{r_1^2 + r_2^2} = (2,219)^2 + (1,573)^2 = 2,719.05$.   Taking each of the smallest
divisions as 200, we see that the chances are more than 9,999 out of 10,000
that the fibers in group *a* are the stronger.   Now, taking h successively
as 5,000, 10,000, 15,000, 20,000, and 25,000, we shall have the quantity
$m_1 - m_2 - h$ taking successively the values 20,887, 15,887, 10,887, 5,887,
and 887.   Taking each of the smallest divisions as 100, we see that the
chances are more than 9,999 out of 10,000 that group *a* is at least 10,000
dgm. per sq. mm. stronger than *b*.   The chances are practically 995
out of 1,000 that group *a* is at least 15,000 dgm. per sq. mm. stronger
than *b*.   The chances are 92 out of 100 that group *a* is at least 20,000
dgm. per sq. mm. stronger than *b* and 6 out of 10 that it is at least 25,000
dgm. per sq. mm. stronger than *b*.   ·Reading these facts in another way,
we may state with the probability of being correct 60 per cent of the
time that the fibers of group *a* are at least 25,000 dgm. per sq. mm.
stronger than those of group *b*; 70 per cent of the time that group *a*
is at least 23,700 dgm. per sq. mm. stronger than group *b*; 75 per cent
of the time that group *a* is at least 23,150 dgm. per sq. mm. stronger than
group *b*; 80 per cent of the time that group *a* is at least 22,500 dgm.
per sq. mm. stronger than group *b*; 85 per cent of the time that group *a*
is at least 21,750 dgm. per sq. mm. stronger than group *b*; 90 per cent
of the time that group *a* is at least 20,700 dgm. per sq. mm. stronger than

group b; 95 per cent of the time that group a is at least 19,250 dgm. per sq. mm. stronger than group b; 99 per cent of the time that group a is at least 15,700 dgm. per sq. mm. stronger than group b; 99.9 per cent of the time that group a is at least 13,500 dgm. per sq. mm. stronger than group b and 99.99 per cent of the time that group a is at least 11,000 dgm. per sq. mm. stronger than group b. We thus read immediately the degree of accuracy to which any measurement is entitled.

As a further illustration, a series of tests were taken on the breaking stresses, diameters, tensile strengths, Young's moduli, and elastic limits of the fibers of samples of wool clipped from the same sheep in successive years, to determine the effect of the age of the sheep on the wool. Taking two of these elements—namely, the breaking stress and elastic limit— it may be shown how accurately we may state the probable tendency of change from year to year (Table IV).

TABLE IV.—*Probable change in the breaking stress and the elastic limit of fibers of wool*

Clip.	Observed.		Probability of decrease in breaking stress.	Observed.		Probability of decrease in elastic limit.
	Breaking stress.	$\sqrt{r_1^2+r_2^2}$		Elastic limit.	$\sqrt{r_1^2+r_2^2}$	
	Dgm.			*Dgm.*		
1908...	64. 22±1. 428			18. 44±0. 2347		
		1. 95	0. 68		0. 36	*a* 0. 87
1909...	62. 91±1. 325·			18. 81± . 2832		
		1. 74	. 79		. 37	. 9998
1910...	60. 81±1. 1286			18. 64± . 2698		
		1. 53	. 9999		. 43	. 52
1911...	52. 03±1. 0792			16. 61± . 3263		
		1. 7	*a*. 9999		. 43	*a*. 69
1912...	68. 12±1. 3871			16. 91± . 2556		
		1. 84	. 9999		. 38	. 9999
1913...	52. 26±1. 248			13.·39± . 2954		

a Increase.

Table shows that in case of the breaking stress the chances are approximately 2 to 1 and 3 to 1 that there was a decrease in the breaking stress from the first to the second and from the second to the third year, respectively, and does not permit us to make a decided statement that there was a decrease those two years.· On the other hand, the chances are better than 10,000 to 1 that from the third to the fourth year and from the fifth to the sixth year there was a decrease as well as from the fourth to the fifth there was an increase in breaking stress. Likewise, we are practically certain that there was a decrease in tensile strength from the second to the third and from the fifth to the sixth years, while in the case of other years the probability is not great enough to justify any very decided statement.

PLATE LVII

Fig. 1.—Diagrammatic drawing of the fiber-testing machine of the Philadelphia Textile School. "*J*, Jaws with screw clamps for holding the fiber; the lower jaw may be raised or lowered; *R*, sliding rod working on a rack and pinion; this takes the place of weights; *G*, wheel graduated on its face in decigrams, moving on the same axis as the pinion for sliding the weight; *T*, thumbscrew for turning the small shaft working the pinion at *P; W*, counterbalancing weight for regulating the zero point of the machine; *S*, scale for reading the sketch of the fiber." (From Matthews' The Textile Fibres.)

Fig. 2.—Fiber-testing machine removed from its case.

Fig. 3.—Diagram showing the arrangement of the wool-testing apparatus at the Montana Agricultural Experiment Station.
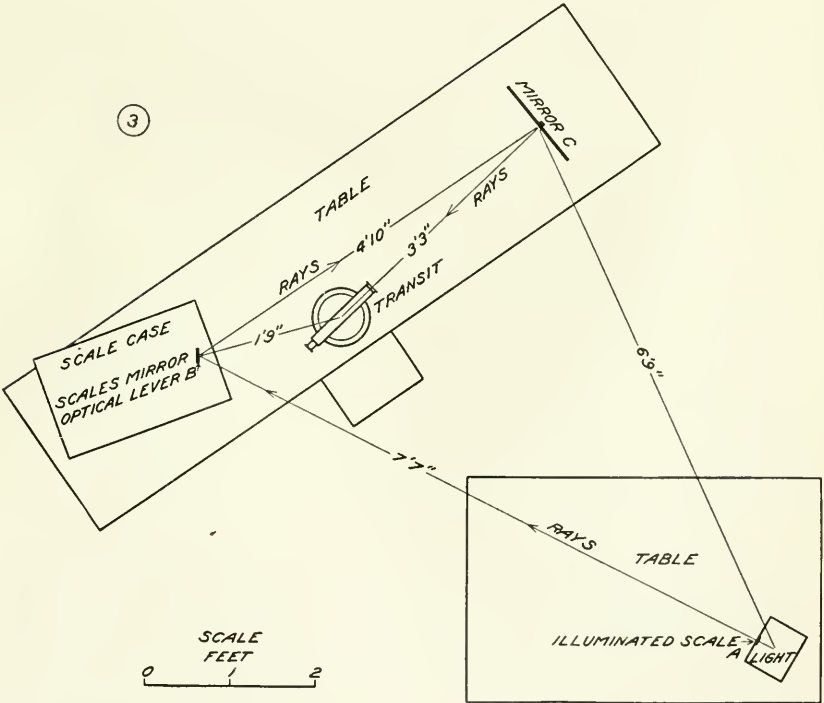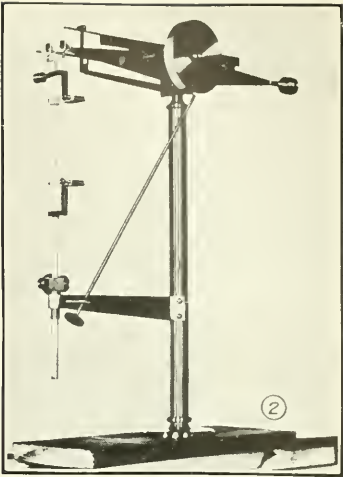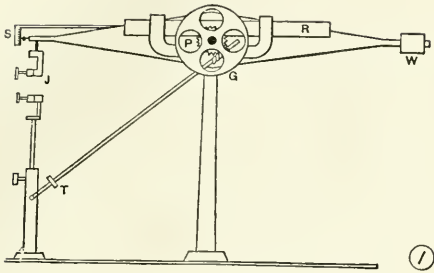
INFLUENCE OF HYBRIDIZATION AND CROSS-POLLINA-
TION ON THE WATER REQUIREMENT OF PLANTS

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INTRODUCTION

In breeding plants for drought resistance it is desirable to know whether the water requirement of the hybrid progeny bears a definite relationship to the water requirement of its parents. That hybridization may result in increased drought resistance is indicated by the work of Collins,[1] who observed that certain first-generation hybrids of maize suffered less from drought than the parents grown under the same conditions. The behavior of these hybrids suggests that they may be exceptionally efficient in the use of water, a point of practical importance in connection with drought resistance. This consideration, combined with the fact that water-requirement measurements constitute a physiological expression of the effects of hybridization, led the writers to measure the water requirement of a number of hybrids and their parents, the subject being one which has not heretofore been quantitatively investigated. These measurements, which were conducted at Akron, Colo., were made possible through the courtesy of Mr. G. N. Collins, of the Office of Crop Acclimatization and Adaptation Investigations, Bureau of Plant Industry, who supplied seed of a number of first-generation hybrids of maize and their parent strains. Mr. J. H. Parker, of the Office of Cereal Investigations, Bureau of Plant Industry, also kindly furnished seed of a hybrid strain of wheat and its parent strains.

The term "water requirement" is here used to designate the ratio of the total weight of water absorbed by the plant during its growth to the total dry matter produced, excluding the roots. The plants were grown in large iron pots of a type already described.[2] To exclude rainfall and prevent evaporation from the soil as far as possible, each pot was provided with a tight-fitting cover having openings for the plants, the annular space between the stalk and the cover being closed with a plastic wax. The plants made a normal growth, as reference to Plate LVIII will show.

[1] Collins, G. N. The value of first-generation hybrids in corn. U. S. Dept. Agr. Bur. Plant Indus. Bul. 191, p. 32. 1910.

[2] Briggs, L. J., and Shantz, H. L. The water requirement of plants. I.—Investigations in the Great Plains in 1910 and 1911. U. S. Dept. Agr. Bur. Plant Indus. Bul. 284, p. 9. 1913.

MAIZE HYBRIDS

In order to make a satisfactory comparison of the water requirement of a hybrid with that of its parents, it is necessary to have the plants growing under as nearly identical conditions as possible. Each determination includes, therefore, the measurement not only of the water requirement of the hybrid but of each parent as well. The work can, of course, be lessened somewhat by the employment of the same parent in more than one combination when such material is available.

The maize hybrids grown in 1912 and 1913 were all from the same female parent, a Chinese type.[1] This is a peculiar corn with a waxy endosperm, received by the Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry, from Shanghai, China. Its water requirement compared with other varieties of maize is relatively high. Various hybrids of this variety were used each year, so that the water requirement of the China type was measured three years in succession. It will be seen that its water requirement in 1913 was much higher than during the two other years. Eleven other species of plants grown at Akron during 1912 and 1913 showed a similar increase in water requirement in 1913, attributable to climatic differences in the two seasons.[2] The variation in water requirement with different seasons does not enter into the present discussion, since only strains that were grown together during the same season are compared.

Of the other varieties tested, Laguna is a Mexican variety grown extensively in Texas, where it has a reputation for drought resistance. Esperanza is a hairy Mexican variety (*Zea hirta* Bonifous) introduced by the Office of Crop Acclimatization and Adaptation Investigations. Hopi is a dwarf variety grown by the Hopi Indians of Arizona, and Pima is a soft corn grown by the Pima Indians at Sacaton, Ariz. Algeria is a slate-colored pop corn the seed of which was imported from Algeria but which came originally from Morocco.[3] Joaquin is an American soft corn from Bradford Island, San Joaquin River, Cal. Budapest is a Hungarian variety of pop corn.

The results of the water-requirement measurements for the individual pots are given in Table I, together with the mean for each series. Six pots were employed in each series in 1912[4] and 1913 and five pots in 1914, which affords a basis for computing the probable error of the mean value obtained.

[1] Collins, G. N. A new type of Indian corn from China. U. S. Dept. Agr. Bur. Plant Indus. Bul. 161, 30 p., 2 pl. 1909.

[2] Briggs, L. J., and Shantz, H. L. Relative water requirement of plants. *In* Jour. Agr. Research, v. 3, no. 1, p. 54. 1914.

[3] Collins, G. N. The value of first-generation hybrids of corn. U. S. Dept. Agr. Bur. Plant Indus. Bul. 191, p. 25. 1910.

——— and Kempton, J. H. Effects of cross-pollination on the size of seed in maize. *In* U. S. Dept. Agr. Bur. Plant Indus. Circ. 124, p. 10. 1913.

[4] With the exception of the Esperanza series, which included only 4 pots.

Table I.—*Water requirement of first-generation hybrids of corn and their parent strains at Akron, Colo., in 1912, 1913, and 1914*

Plant and period of growth.	Pot No.	Dry matter.	Water.	Water require-ment based on dry matter.
1912.		*Gm.*	*Kg.*	
	289	376. 6	112. 4	298
	290	261. 2	83. 8	321
Laguna (*Zea mays*), July 2 to Sept. 26..........	291	268. 9	84. 0	313
	292	448. 1	124. 4	278
	293	457. 4	127. 8	279
	294	429. 2	119. 4	278
Mean..........................	....	374±27	....	295±6
	271	790. 5	224. 9	284
	272	500. 8	152. 2	304
China × Laguna (*Zea mays*), June 12 to Sept. 26..................	273	514. 0	145. 6	283
	274	615. 0	179. 2	291
	275	571. 5	172. 8	302
	276	690. 0	187. 5	272
Mean..........................	....	614±32	....	289±4
	265	243. 5	84. 3	346
	266	577. 0	184. 6	320
China (*Zea mays*), June 12 to Sept. 26..........................	267	319. 0	97. 1	304
	268	524. 5	173. 9	331
	269	660. 9	179. 6	272
	270	401. 5	126. 1	314
Mean..........................	....	454±44	....	315±7
	259	634. 6	151. 2	238
	260	638. 4	161. 3	253
China × Esperanza (*Zea mays*), June 12 to Sept. 26..................	261	582. 8	149. 8	257
	262	515. 5	133. 0	258
	263	583. 7	146. 5	251
	264	764. 0	186. 1	244
Mean..........................	....	618±22	....	250±2
	301	492. 3	114. 3	232
Esperanza (*Zea mays*), June 12 to Sept. 26..........................	302	574. 7	133. 7	233
	303	563. 7	141. 7	252
	304	510. 7	122. 7	240
Mean..........................	....	536±17	....	239±3
1913.				
	313	346. 7	118. 8	343
	314	400. 0	135. 8	340
Hopi (*Zea mays*), June 14 to Sept. 16.	315	472. 5	170. 2	360
	316	417. 1	147. 0	352
	317	405. 3	163. 4	403
	318	619. 6	185. 4	300
Mean..........................	....	444±27	....	350±8

TABLE I.—*Water requirement of first-generation hybrids of corn and their parent strains at Akron, Colo., in 1912, 1913, and 1914*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Water.	Water require-ment based on dry matter.
1913.		*Gm.*	*Kg.*	
	307	757. 2	254. 5	336
	308	706. 5	257. 2	364
Hopi×China (*Zea mays*), June 7 to Sept. 16	309	712. 2	245. 5	345
	310	728. 1	247. 2	340
	311	715. 2	246. 8	345
	312	716. 6	242. 0	338
Mean		723±5		345±3
	301	554. 9	228. 2	411
	302	487. 5	210. 4	432
China (*Zea mays*), June 7 to Sept. 16.	303	492. 6	202. 3	411
	304	589. 2	228. 1	387
	305	478. 7	198. 7	415
	306	523. 1	226. 6	433
Mean		521±13		415±4
	295	661. 3	257. 9	390
China (*Zea mays*) × Teosinte (*Eu-chlaena mexicana*), June 7 to Sept. 16	296	613. 2	228. 9	373
	297	657. 9	239. 7	364
	298	654. 0	239. 3	366
	299	607. 5	237. 8	391
	300	651. 7	244. 0	374
Mean		641±17		376±4
	289	616. 4	234. 7	380
	290	534. 5	211. 6	395
Teosinte, Durango (*Euchlaena mexi-cana*), June 14 to Sept. 16	291	624. 5	194. 0	311
	292	567. 3	231. 5	408
	293	520. 0	214. 4	412
	294	421. 4	183. 2	434
Mean		547±21		390±11
1914.				
	238	354. 7	117. 8	332
	239	521. 2	161. 1	309
Algeria (*Zea mays*), June 3 to Aug 31.	240	431. 3	149. 0	346
	241	392. 0	131. 4	335
	242	386. 7	127. 8	331
Mean		417±20		331±4
	243	515. 0	176. 3	342
	244	474. 2	175. 0	369
Algeria × China (*Zea mays*), June 3 to Aug. 31	245	524. 4	178. 3	340
	246	573. 0	187. 6	328
	247	477. 5	170. 9	358
Mean		512±15		347±5

TABLE I.—*Water requirement of first-generation hybrids of corn and their parent strains at Akron, Colo., in 1912, 1913, and 1914*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Water.	Water requirement based on dry matter.
1914.		*Gm.*	*Kg.*	
	248	401. 3	149. 2	372
	249	313. 9	104. 2	332
China (*Zea mays*), June 3 to Aug. 31..	250	394. 7	130. 9	332
	251	406. 7	130. 1	320
	252	419. 3	143. 1	341
Mean..........................	....	387±12	....	338±6
	253	343. 3	140. 7	410
	254	287. 0	91. 3	318
Joaquin (*Zea mays*), June 3 to Aug. 31..............................	255	*a* 96. 3	35. 1	364
	256	201. 7	74. 7	370
	257	301. 0	113. 6	378
Mean..........................	....	283±17	....	368±9
	258	410. 3	142. 4	347
	259	453. 5	159. 4	352
Budapest × Joaquin (*Zea mays*), June 3 to Aug. 31..............	260	382. 7	148. 9	389
	261	358. 0	131. 8	368
	262	388. 4	143. 4	369
Mean..........................	....	399±11	....	365±4
	263	427. 5	150. 2	351
	264	398. 3	134. 4	337
Budapest (*Zea mays*), June 3 to Sept. 1......................	265	451. 7	150. 8	334
	266	379. 7	133. 2	351
	267	396. 7	139. 3	351
Mean..........................	....	411±10	....	345±4
	268	368. 4	137. 4	373
	269	364. 8	149. 8	411
Budapest × Pima (*Zea mays*), June 3 to Sept. 1.....................	270	362. 6	144. 1	398
	271	364. 2	138. 4	380
	272	348. 5	132. 8	381
Mean..........................	....	362±2	....	389±5
	273	350. 7	134. 0	382
	274	343. 0	132. 0	385
Pima (*Zea mays*), June 3 to Sept. 1..	275	375. 3	123. 2	328
	276	335. 2	120. 5	360
	277	402. 3	149. 6	372
Mean..........................	....	361±9	....	365±7
	278	365. 5	151. 4	414
	279	388. 9	161. 2	415
Joaquin × Pima (*Zea mays*), June 3 to Sept. 1......................	280	352. 3	126. 8	360
	281	324. 9	121. 3	374
	282	433. 3	165. 2	381
Mean..........................	....	373±13	....	389±9

a Omitted in calculating the mean.

The results are summarized in Table II, the female parent being given first in each instance. In this table is included also the average water requirement of the two parents, together with the ratio of the water requirement of the hybrid to that of the parental mean. The divergence of the parents—i. e., the ratio of their water requirements—is also given in the last column of the table.

TABLE II.—*Water requirement of hybrid and parent strains of corn*

Year and parent strain.	Water requirement based on dry matter.			Ratio of hybrid to parental mean. $\frac{2c}{a+b}$	$\frac{a}{b}$
	Parent strain.		Hybrid.		
	Observed.	Mean.	Observed.		
1912.					
China.....................	315±7	305±5	289±4	0.95±.02	1.07
Laguna....................	295±6				
China.....................	315±7	277±4	250±2	.90±.01	1.32
Esperanza.................	239±3				
1913.					
China.....................	415±4	383±5	345±3	.90±.02	1.19
Hopi......................	350±8				
China.....................	415±4	403±6	376±4	.93±.02	1.07
Teosinte..................	390±11				
1914.					
Algeria...................	331±4	335±4	347±5	1.04±.02	1.02
China.....................	338±6				
Budapest..................	345±4	357±5	365±4	1.02±.02	1.07
Joaquin...................	368±9				
Budapest..................	345±4	355±4	389±5	1.10±.02	1.06
Pima......................	365±7				
Joaquin...................	368±9	367±6	389±9	1.06±.03	1.01
Pima......................	365±7				

Reference to Table II will show that the parents of the hybrids grown in 1912 and 1913 differed in water requirement much more than the parent strains employed in 1914. Each first-generation hybrid of maize grown during the first two years gave a water requirement ranging from 5 to 10 per cent below the mean of its parents. All the maize hybrids grown in 1914 gave a water requirement from 2 to 10 per cent above the mean of the parents. The results of the third year are therefore opposed in direction to those obtained during the first two years.

The parents of the hybrids used in 1912 and 1913 also showed much greater divergence in water requirement than those employed in the 1914 measurements. The question therefore arises as to whether the divergence of the parents may not be a factor in determining the relation of the water requirement of the hybrid to the parental mean.

If we represent the water requirement of the less efficient parent by a, the more efficient parent by b, and the hybrid by c, then the divergence of the parents may be represented by the ratio $\dfrac{a}{b}$, and the divergence of the hybrid from the mean of its parents by the ratio $\dfrac{2c}{a+b}$. These ratios are given in Table II. Plotting these values for the eight hybrids under discussion, we obtain a graph of the form given in figure 1. This graph
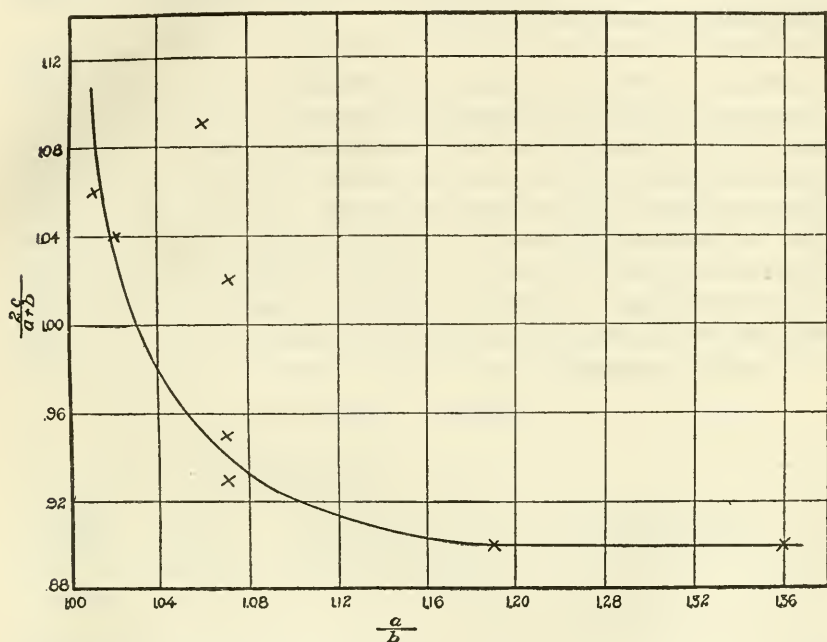
Fig. 1.—Graph showing relation between the parental divergence $\left(\dfrac{a}{b}\right)$ and the hybrid divergence from the parental mean $\left(\dfrac{2c}{a+b}\right)$, where a= water requirement of less efficient parent; b= water requirement of more efficient parent; c= water requirement of hybrid.

indicates a correlation between the divergence of the parents and the divergence of the water requirement of the hybrid from the parental mean. There are two outstanding points on this graph, both associated with Budapest pop corn, which in both hybrids is the more efficient parent. If the water requirement of Budapest variety were slightly increased, the points would tend to approach the graph. On the other hand, four of the eight hybrids under consideration have approximately the same parental mean. Therefore, while the data indicate the existence of a relationship between parental divergence and hybrid divergence, this relationship can be expressed quantitatively only by the use of statistical methods which require many more measurements than are now available and more than the practical importance of the subject would justify at this time.

The mean ratio of the water requirement of each hybrid to the average water requirement of its parents is 0.99±0.01. The probable error here refers to the uncertainty of the individual ratios. In using the data as a basis for predicting the probable departure of a hybrid from this ratio, the observed departures must be considered—i. e., the probable error of a single determination must be computed, which is found in this case to be ±0.06. In other words, according to the data at hand, the chances are that the water requirement of a hybrid will not depart from the mean of its parents by more than 6 per cent.

The relative amount of dry matter produced by hybrids and parents is a point of interest in connection with the relative water requirement.[1] In 1912 and 1913 the hybrids produced from 20 to 50 per cent more dry matter than the mean production of the parent strains (Table III). In 1914 three of the hybrids showed an increase in dry matter compared with the parental mean, and one showed a decrease. An analysis of the individual pot yields for both parents and hybrids shows that the pots giving the greatest weight of dry matter usually have a water requirement below the mean of the strain. The greater vegetative vigor of the maize hybrids may therefore be correlated to some extent with the observed reduction in the water requirement below the parental mean.

TABLE III.—*Dry matter produced by hybrid and parent strains of corn*

Year and parent strain.	Mean dry matter.			Ratio of hybrid to parental mean.
	Parent strain.		Hybrid.	
	Observed.	Mean.	Observed.	
1912.				
China............................	454±44	414±26	614±32	1.48±.12
Laguna...........................	374±27			
China............................	454±44	495±24	618±22	1.25±.08
Esperanza........................	536±17			
1913.				
China............................	521±13	483±15	723±5	1.50±.05
Hopi.............................	444±27			
China............................	521±13	534±13	641±17	1.20±.04
Teosinte.........................	547±21			
1914.				
Algeria...........................	417±20	402±12	512±15	1.27±.05
China............................	387±12			
Budapest.........................	411±10	347±10	399±11	1.15±.05
Joaquin..........................	283±17			
Budapest.........................	411±10	386±7	362±2	.94±.02
Pima.............................	361±9			
Joaquin..........................	283±17	322±10	373±13	1.16±.05
Pima.............................	361±9			

[1] Collins has called attention to the marked increase in yield often noted in first generation hybrids of maize. (Collins, G. N. The value of first-generation hybrids in corn. U. S. Dept. Agr. Bur. Plant Indus. Bul. 191, p. 32. 1910.)

The data presented indicate, so far as they are representative of first-generation maize hybrids as a class, that striking differences between the water requirement of hybrids and the mean water requirement of the two parents are not to be expected. The greatest observed departure of the hybrid from the parental mean was ±10 per cent, and, according to the available measurements, the chances are even that first-generation maize hybrids will not depart more than ±6 per cent from the parental mean. This departure, moreover, may take place in either direction— i. e., the hybrid may resemble either parent as regards efficiency in the use of water.

In investigations of this kind more extensive measurements are always desired, both by the reader and the author. To determine with more precision the correlation between hybrids and parents as regards water requirement would necessitate a sufficiently large number of determinations to justify the use of statistical methods. The expense and labor involved in such measurements is great, each determination necessitating the care of from 15 to 18 pots of plants throughout the growing season. Since the results already obtained indicate that hybrids depart but slightly from the mean water requirement of their parents, more extended determinations are not believed to be justified at the present time.

WHEAT HYBRID

The wheat hybrid used was a cross between *Triticum durum* and *Triticum aestivum*. This hybrid strain has been grown for some generations and shows no increase in vegetative vigor as compared with the parent strains. The dry matter produced was practically uniform for parents and hybrid, but the grain yield of the hybrid was below its parents, and this further increases the water requirement of the hybrid when based on grain production.

Reference to Table IV will show that the water requirement of the hybrid is decidedly above both parents (14±1 per cent above the parental mean).

TABLE IV.—*Water requirement of parent and hybrid strains of wheat in 1914*

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirements based on—	
						Grain.	Dry matter.
		Gm.	*Gm.*	*Kg.*	*P. ct.*		
Wheat, Iumillo, C. I. 1736 (*Triticum durum*), May 23 to Aug. 11.	67	209. 7	83. 0	110. 4	39	1,330	526
	68	240. 7	91. 7	129. 0	38	1,406	536
	69	252. 5	92. 7	120. 5	37	1,300	477
	70	265. 7	98. 6	134. 8	37	1,367	508
	71	262. 3	95. 5	125. 0	36	1,309	477
	72	334. 8	132. 1	151. 8	39	1,148	454
Mean..........	......	261±9	......	....	......	1,310±21	496±10

TABLE IV.—*Water requirement of parent and hybrid strains of wheat in 1914*—Contd.

Plant and period of growth.	Pot No.	Dry matter.	Grain.	Water.	Grain.	Water requirements based on—	
						Grain.	Dry matter.
		Gm.	Gm.	Kg.	P. ct.		
Wheat, I u m i l l o × Preston, May 19 to Aug. 1.	73	283. 6	75. 0	162. 3	26	2, 162	572
	74	302. 2	91. 2	172. 6	30	1, 895	571
	75	253. 6	72. 1	147. 6	28	2, 050	582
	76	225. 8	65. 6	127. 9	29	1, 948	567
	77	226. 6	67. 5	129. 6	29	1, 921	572
	78	235. 9	70. 2	136. 6	29	1, 947	580
Mean.........	.....	255±13	.....	.....	.....	1,987±31	574±2
Wheat, Preston, C. I. 3328 (*Triticum aestivum*), May 23 to Aug. 3.	79	237. 4	78. 4	118. 0	33	1, 505	497
	80	261. 3	81. 1	132. 9	31	1, 638	508
	81	281. 4	94. 7	147. 8	34	1, 561	525
	82	215. 8	75. 5	110. 7	35	1, 466	514
	83	249. 2	87. 0	129. 6	35	1, 490	520
	84	224. 3	79. 4	111. 7	35	1, 407	498
Mean.........	.....	245±10	.....	.....	.....	1, 511±22	510±3

EFFECT OF SELF- AND CROSS-POLLINATION

In addition to the maize hybrids Mr. Collins also supplied self-pollinated seed of two individuals, together with cross-pollinated seed from the same individuals. The cross-pollinated plants in this case represent pure seed of the selected strain. Reference to Table V will show that in one instance self-pollination produced no measurable change in the water requirement, while in the other instance an increase in water requirement of 4±1 per cent was observed. The plants from the cross-pollinated seed also gave a higher yield of dry matter. The effect of cross-pollination between individuals is, in this instance at least, quite similar to results produced by the cross-pollination of different strains, so far as water requirement and yield are concerned.

TABLE V.—*Effect of self- and cross-pollination on the water requirement of corn in 1914*

Plant and period of growth.	Pot No.	Dry matter.	Water.	Water requirement based on dry matter.
		Gm.	Kg.	
Corn, German, C24–1 (*Zea mays*), June 3 to Sept. 1.	223	354. 4	135. 4	382
	224	296. 7	107. 5	363
	225	333. 2	129. 8	390
	226	306. 4	122. 4	399
	227	317. 4	125. 1	394
Mean......................	.....	322±8	....	386±3

TABLE V.—*Effect of self- and cross-pollination on the water requirement of corn in 1914*—Continued

Plant and period of growth.	Pot No.	Dry matter.	Water.	Water requirement based on dry matter.
		Gm.	*Kg.*	
	228	419. 0	155. 6	372
Corn, German, C24–1x2 (*Zea mays*), June 3	229	432. 4	158. 2	366
to Sept. 1.	230	362. 2	136. 3	376
	231	357. 0	133. 7	375
	232	379. 5	140. 4	370
Mean...........................	....	390±12	....	372±1
	233	374. 6	143. 8	384
Corn, German, C24–2 (*Zea mays*), June 3	234	407. 5	148. 2	364
to Aug. 31.	235	399. 8	150. 0	376
	236	379. 7	139. 9	368
	237	335. 0	123. 2	368
Mean...........................	....	379±9	....	372±2

CONCLUSIONS

Eight first-generation hybrids of maize and one wheat hybrid, together with their parent strains, were included in water-requirement measurements at Akron, Colo., from 1912 to 1914. The hybrids ranged in water requirement from 10 per cent below to 10 per cent above the parental mean. On the basis of the results so far obtained, the chances are even that a maize hybrid will not depart in its water requirement more than ±6 per cent from the parental mean.

Cross-pollination between individual plants of maize leads to results similar to hybridization of different strains, so far as water requirement and yield are concerned.

A wheat hybrid which had been grown for several generations gave a water requirement 14 per cent above the mean water requirement of the parental strains.

First-generation hybrids and parents used in 1912 experiments. The lower leaves of some of these varieties had already matured at the time the photographs were taken and had been picked and placed in the bags attached to the pots.

Fig. 1.—Laguna corn (pots 289–294), grown July 2 to September 26, 1912. Photographed on September 9, 1912. Water requirement, 295±6.

Fig. 2.—Esperanza corn (pots 301–304), grown June 12 to September 26, 1912. Photographed on September 7, 1912. Water requirement, 239±3.

Fig. 3.—China corn (pots 265–270), grown June 12 to September 26, 1912. Photographed on September 9, 1912. Water requirement 315±7.

Fig. 4.—Hybrid China × Laguna corn (pots 271–276), grown June 12 to September 26, 1912. Photographed on September 9, 1912. Water requirement 289±4.

Fig. 5.—Hybrid China × Esperanza corn (pots 259–264), grown June 12 to September 26, 1912. Photographed on September 7, 1912. Water requirement, 250±2.

PLATE LVIII

FURTHER STUDIES OF THE EMBRYOLOGY OF TOXOPTERA GRAMINUM

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In 1912 the writer, as junior author,[1] gave in a general way an account of the development of the winter egg of *Toxoptera graminum* Rondani. This is the first time in this country that the development of the winter egg of any species of Aphididae has been followed out beyond the early stages. It was recognized at the time that there was a wide gap in the continuity of the study of this development, which was not represented by the material then at hand. This paper is intended to supply briefly this missing link.

A few eggs were collected in the fall of 1911 and the development was watched carefully in the spring of 1912. This material gave positive evidence that several links were missing in the data previously obtained. Bulletin 110 of the Bureau of Entomology was then in press, and it was too late to do more than indicate where the additional data belonged. As the amount of material obtained in 1911 was rather limited, a large number of eggs were collected in the fall of 1912 for further study. As previously stated,[2] no attempt has been made to treat the subject exhaustively, only the main points in the development being considered.

The same methods of fixation, staining, etc., described in the previous paper [3] were employed.

Turning to Plate VII of Bulletin 110, one will readily see that the gap previously referred to occurs between figures 1 and 2, where the polar organ is entirely lost track of after figure 1. It is also at this point in the development of the embryo that the revolution occurs; hence, there is not a single figure in the first publication to illustrate the revolution of the embryo and the fate of the polar organ.

The polar organ is a unique, newly discovered body, since, so far as the author's information goes, no other observer has heretofore figured any such body; and, while it was exceedingly unfortunate that figures illustrating its fate and the revolution of the embryo could not then be supplied, this serious defect is now eliminated. In view of the necessarily incomplete Plate VII of Bulletin 110, it has been thought best to make an entirely new series of figures covering the same period of development as that covered by Plate VII, drawn from a new series of sections,

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[1] Webster, F. M., and Phillips, W. J. The spring grain aphis or "green bug." U. S. Dept. Agr. Bur. Ent. Bul. 110, 153 p., 48 fig., 4 diagr., 9 pl. 1912.

[2] Id., p. 94.

[3] Id., p. 95.

all of which were cut at the same angle, in this way rendering the continuity more uniform and more easily apparent. The following discussion chiefly relates to the revolution of the embryo, which for reasons already given it was impossible to include in the previous publication.

As the embryo starts from the yolk it approaches the posterior pole of the egg until the amnion in the dorso-cephalic region comes in contact with the serosa, as shown in Plate LIX, figure 1, of this paper. These membranes then unite and the embryo moves forward slightly, as in Plate VII, figure 2.[1]

When the amnion and serosa are in contact, the central cavity of the polar organ still opens upon the surface of the egg. This cavity is filled with some substance that does not take the stains so far used. It is at this time that it is very difficult to remove the shell without also removing the contents of the cavity en masse, as they appear to adhere to the shell. In later stages the cavity is empty and has no opening upon the surface of the egg. From this it would appear that the polar organ does act in an excretory capacity and that its entire contents are eliminated when the embryo begins its revolution, and that it ceases to function after this time.

Figure 3 (Pl. LIX) is slightly more advanced, and it is very apparent that the embryo is starting its revolution. The greater portion of the yolk has now collected at the opposite pole of the egg, and the mesenteron is complete throughout.

Figures 4 and 5 (Pl. LIX) represent the embryo much crumpled and folded upon itself in the act of making its revolution, occupying the entire posterior part of the egg, the yolk having collected in the anterior region. The polar organ is migrating backward. Figure 6 (Pl. LIX) illustrates the embryo after the turn is completed, and the polar organ is on the opposite side of the egg. It will be noted in this figure that the cells are crowding together anterior to the polar organ.

Development from figures 1 to 6 (Pl. LIX) progresses very rapidly, so much of the revolution being accomplished in a few hours. To obtain these stages it was necessary to fix and examine large numbers of eggs every few hours.

Figures 1 to 3, Plate LX, show more advanced stages of the revolution of the embryo and are especially interesting from the fact that they illustrate the fate of the polar organ. It is very apparent that it merges with and loses its identity in the large mass of cells that accumulate in the cephalic region after the revolution of the embryo, which later forms the dorsal organ.

Figures 4 to 6, Plate LX, represent the fate of the dorsal organ after its previous fusion with the polar organ. Under favorable weather conditions the insect will hatch very soon after this point in the development has been reached.

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[1] Plate VII, figure 1, of U. S. Dept. Agr. Bur. Ent. Bul. 110 is probably a little misleading, in that it would appear as though the serosa and amnion came in contact in the region of the ventral part of the head, which is not true.

PLATE LIX

Toxoptera graminum:

Fig. 1-3.—Sagittal sections. Embryo starting revolution. Note changing position of the polar organ, shown at *p. o.* × 83.

Fig. 4-6.—Sagittal sections. Embryo making revolution. Note polar organ, shown at *p. o.*, migrating backward. × 83.
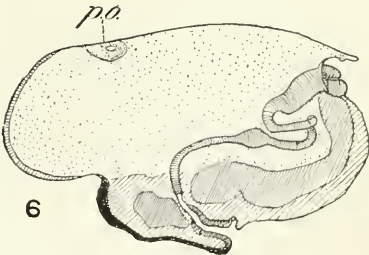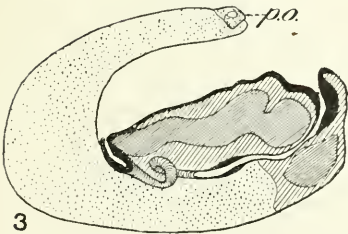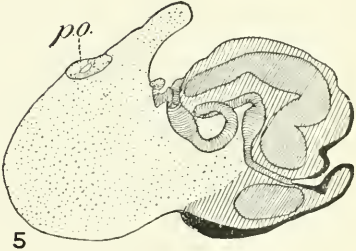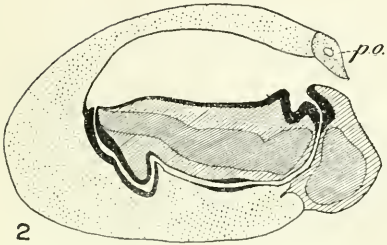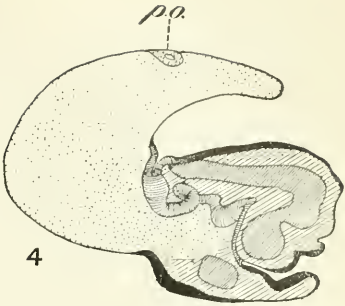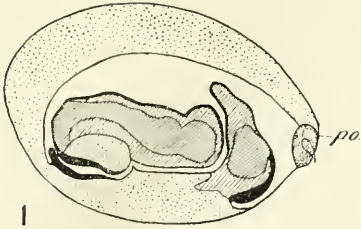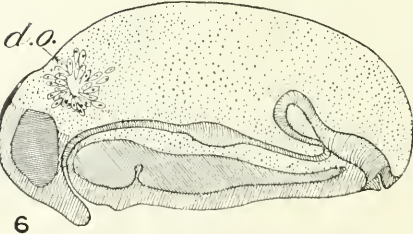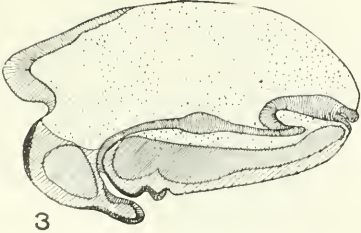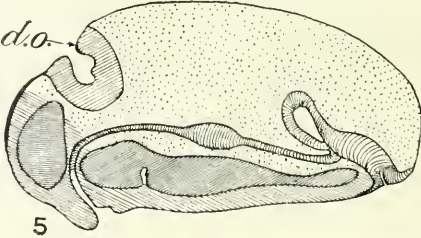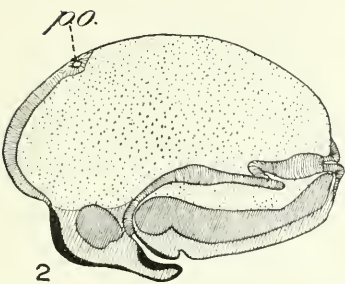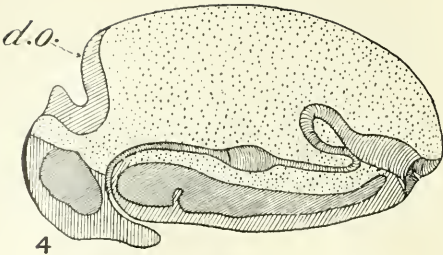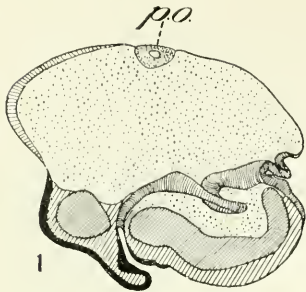
PLATE LIX

PLATE LX

Toxoptera graminum:

Fig. 1–3.—Sagittal sections.   Revolution is almost complete and shows fate of the polar organ, *p. o.*   ✕ 83.

Fig. 4–6.—Sagittal sections.   *d. o.*, Dorsal organ.   ✕ 83.

PRICKLY-PEARS AS A FEED FOR DAIRY COWS

By T. E. Woodward, *Dairy Husbandman*, and W. F. Turner, *Assistant Dairy Husbandman, Bureau of Animal Industry*, and David Griffiths, *Agriculturist, Office of Farm Management, Bureau of Plant Industry*

INTRODUCTION

Prickly-pears (*Opuntia* spp.) have been fed to cattle for many years in Texas and Mexico, but they have formed only a small part of the ration, and their value as a feed has not been fully appreciated. In the wild state these cacti make a rank growth, and experiments show that they respond readily to cultivation, two years' growth from old stumps yielding as high as 106 tons an acre a year. The average annual yield at Brownsville, Tex., under ordinary cultural conditions for the first two years' growth from cuttings was about 40 tons and at San Antonio, Tex., about 25 tons an acre. The second two years' growth from the cut-over stumps would be still larger. As irrigation of these plants is unnecessary, the cost of growing the crop is very low considering the tonnage produced; and although prickly-pears contain about 90 per cent of water, the production of dry material is large. Since there are no doubts as to the practicability of growing prickly-pears as a farm crop, the only other vital consideration is their feeding value. If it can be shown that they possess sufficient nutrients and have no injurious effect on the animals, there is no reason why these cacti should not come into general use in all sections where they can be readily grown.

One of the writers (Griffiths, 5)[1] conducted a feeding trial for 67 days with two cows at the ranch of Mr. Alexander Sinclair, San Antonio, Tex., and found that these cacti were a palatable and nutritious feed for short periods and that the flavor of the milk was in no way impaired. He also reported the feeding of 20 steers on the ranch of Mr. T. A. Coleman, Encinal, Tex., with a ration of prickly-pear and cottonseed meal for 105 days. Each pound of gain required 55.03 pounds of prickly-pear and 2.5 pounds of cottonseed meal, which was a very satisfactory showing.

Hare (7), of New Mexico, conducted five digestion trials, with two steers in each trial, in which he showed that when the prickly-pear was fed with cured fodders or with grains, the digestibility of both was increased. The five rations that he used were as follows: Prickly-pear (*O. lindheimeri*), prickly-pear (*O. laevis*), prickly-pear and alfalfa, prickly-pear and cottonseed meal, and alfalfa hay.

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[1] Reference is made by number to "Literature cited," p. 434.

Sotgia (10) reported an experiment on feeding prickly-pear to five cows in Sardinia which indicated in general that the prickly-pear increases the milk yield without lowering the percentage of solids in the milk.

On the other hand, Mr. P. R. Mehta (9), Acting Deputy Director of Agriculture, Bombay Presidency, India, in reporting the results of a feeding trial conducted with five animals for five months in 1902, stated:

> The result of our extended and thorough trial proves conclusively that prickly-pear has hardly any value as a cattle feed. It is only when given with a moderate quantity of ordinary fodder that the animals can just manage to live for a period of four to five months.

While these and other interesting and valuable observations on the feeding of the plant have been made, it was thought advisable to obtain more definite knowledge than is afforded by the ordinary feeding practice. Accordingly, the Dairy Division of the Bureau of Animal Industry, cooperating with the Office of Farm Management of the Bureau of Plant Industry, conducted the experiments herein reported at the South Texas Gardens, Brownsville, from October, 1911, to April, 1913.

PLAN OF THE INVESTIGATIONS

CULTIVATION OF PRICKLY-PEAR

The prickly-pears used in these experiments consisted of cuttings of native species collected from uncultivated lands near Brownsville and set out in the cultivated fields of the experimental farm. The stock which it was thought would produce the largest yields was used in the plantings. Three species common to the region were planted, *O. gommei*, *O. cyanella* (color Pl. F), and an unnamed variety. All were probably confined in their distribution to the delta region of the Rio Grande. Although these species differ botanically there is very little distinction between their forage value and that of many other spiny species in southern Texas, except that these delta species are the most viciously spiny and the most difficult to singe. The difficulty in singeing is caused mainly by the large number of coarse spines and the still larger number of long, coarse spicules; and the region being near the coast, where the atmosphere is humid, the spines of all species are less combustible.

The two varieties named constituted probably 95 to 98 per cent of the plantation. The third species differed from these in several particulars, but more especially in its habit of growth. It is judged to be of less value than either of the two others, mainly on account of its smaller, thinner joints. However, this species did not form more than from 3 to 5 per cent of the field.

The main crop of prickly-pear was planted in the spring about 18 months before the feeding was begun and consequently had had two seasons' growth. As the feeding was continuous from the date of beginning,

the material used in these experiments represented the growth of the first, second, and third seasons. The ground was put in a moderate state of tilth, and single-jointed cuttings were set about 30 inches apart in rows. In previous work of the Department at San Antonio and elsewhere, the rows were 6 feet apart, but on account of the greater rapidity of growth at Brownsville, this distance was increased to 8 feet. Moderate cultivation was given for the first year and the early part of the second; but after August of the second season little cultivating could be done, and after September cultivation was abandoned because the plants filled the space between the rows, preventing the horses from passing.

The yields of prickly-pear obtained in this field were not at all typical of what may be expected in this delta region. The plot of ground used was badly infested with Bermuda grass, was flooded considerably during heavy rains, and had three depressions of very stiff Cameron clay running through it. These conditions very materially reduced the yields.

OUTLINE OF THE FIRST YEAR'S TESTS

In selecting materials for use in comparison with prickly-pear, such feeds were chosen as are common to its growing region. It was thought best, therefore, to compare the value of prickly-pear with sorghum hay, sorghum silage, and cottonseed hulls, since these are the feeds which these cacti might replace either wholly or in part. In selecting the animals an effort was made to secure mature but not aged cows that gave evidence of being at least fair milk producers. Accordingly, 13 grade Jersey cows that had been fresh but a few weeks were purchased near Brownsville (Pl. LXII, fig. 2). They were somewhat better than the ordinary Texas dairy stock, and were accustomed to eating prickly-pear. These cows were grouped and fed as shown in Table I.

TABLE I.—*Grouping and rations of cows in first year's tests* [a]

Number of cows in group.	Ration.	
	First period (80 days).	Second period (80 days).
3	Grain, hay, and heavy prickly-pear	Grain, hay, and medium prickly-pear.
3	Grain, hay, and medium prickly-pear	Grain, hay, and heavy prickly-pear.
3 [b]	Grain, hay, and medium prickly-pear	Grain and hay.
3	Grain and hay	Grain, hay, and medium prickly-pear.
1	Cottonseed meal and heavy prickly-pear.	

[a] There was a transition period of 10 days between the two 80-day periods.
[b] One of the cows in this group died; the data for this cow have been disregarded in calculating the results.

The first and second groups were for the comparison of medium and large quantities of prickly-pear as well as the relative values of hay and prickly-pear; the third and fourth groups were for comparing the rela-

tive value of the hay and prickly-pear and the effect of adding prickly-pear to a ration of dry material.

The groups to be compared were balanced as nearly as possible with reference to body weights and yields of milk. In order to do this, records of the milk and fat were kept for a period of 10 days before the cows were divided into groups. The 80-day experimental periods were divided into subperiods of 10 days each, and 10 days were allowed for making the changes in rations at the end of the first 80 days.

The grain mixture consisted of equal parts by weight of corn chop, wheat bran, and cottonseed meal; the hay was sorghum hay of average quality, and the prickly-pear was a very spiny two-years' growth. The prickly-pear was singed in the field with a gasoline torch, then cut off near the ground, and hauled to the cow lot. After being placed in the mangers, the heavier stems were chopped into small pieces with a sharp spade or hoe. The grain was fed according to the amount of milk fat produced, about 10 pounds being supplied for each pound of fat. The milk fat rather than the milk itself, the milk solids, or the energy value of the milk was taken as the basis of feeding, as previous investigations (2) have shown it to be a more reliable guide for this purpose than the whole milk, and perhaps just as reliable as the milk solids or energy value.

As much prickly-pear was fed to the cows of the heavy ration prickly-pear group as they would eat; this varied with the different individuals from 100 to 150 pounds a day. Each cow in the two groups receiving the medium prickly-pear ration received 60 pounds a day, and as much hay was fed to the cows of the four groups as they would consume without undue waste. The quantity varied from 3.5 to 20 pounds, depending upon the individual and the amount of prickly-pear in the ration. In addition to these four groups, one cow received all the prickly-pear she would eat and, in addition, 4 pounds of cottonseed meal daily. The object in this case was to ascertain the physiological effects of feeding large quantities of prickly-pear for a long period.

The body weights of all the cows were controlled by reducing or increasing the roughage so that the gains or losses in weight of the groups to be compared were approximately the same. By conducting the experiment in this manner many of the variable factors were controlled and a direct comparison of the amounts of prickly-pear and hay required to produce a pound of milk fat was made possible.

The body weight of each cow was taken every morning at about the same hour, so that the conditions from day to day as regards fill both of feed and water were as nearly uniform as possible.

The milk was weighed at each milking, and composite samples for fat analysis and specific-gravity determinations were taken for 5 days near the middle of each 10-day period, the samples being preserved with formaldehyde. The milk solids were estimated from the fat and

specific gravity by using Farrington and Woll's table (3, p. 264, tab. 6). At the end of each 10-day period the yield of the milk of each cow was totaled, and the number of pounds of fat for the period was determined. This yield of fat was then used as the basis for computing the grain ration to be fed for the next 10 days.

Every 20 days samples of the ordinary prickly-pear, chopped and thoroughly mixed, were put into quart jars (chloroform being used as a preservative) and sent to the Dairy Division for analysis by Mr. R. H. Shaw.

While this method of determining the relative values of feeds is by no means perfect, it is an improvement on the ordinary feeding experiment, because the factor of live weight was controlled and a direct comparison of the feeds in question made possible on the basis of fat production. It would be well, too, if a comparison could be made on a basis other than that of fat; this, however, owing to the variation in the composition of the milk caused by the feed, is impossible without conducting more experiments. The alternating system of feeding, such as was used in this experiment, favors the poorer feed. If any feed under comparison has a tendency to stimulate production more than another, the cows which receive the better feed for the second period are placed at a disadvantage, as they must begin the second period at a lower level of production than the cows that are to receive the poorer feed. The feeding periods were continued long enough to reduce the experimental error to an insignificant factor.

Cow 7, in the group receiving grain, hay, and the medium prickly-pear ration, died from acute indigestion, but in the opinion of all those connected with the experiment its death can not be ascribed to the prickly-pear any more than to the other ingredients of the ration. In calculating the results of the experiment, therefore, the data for this animal were disregarded.

OUTLINE OF THE SECOND YEAR'S TESTS

As the first-year's work had shown that prickly-pear fed in medium amounts gave best results, it was fed the second year at the rate of 75 pounds a day to each cow in the groups that were used for comparing prickly-pear with other feeds. Two groups were fed to compare the relative values of prickly-pear and the sorghum silage and two to compare the relative values of these cacti and cottonseed hulls; one additional cow was fed to study the effect of prickly-pear when fed for a long period; another cow was fed on prickly-pear without any supplementary ration to ascertain whether it would be possible to maintain an animal upon this feed alone. The cottonseed hulls were such as are ordinarily purchased in the open market. The sorghum silage was below the average in quality, as the sorghum had been sown broadcast and was not fully mature at the time of putting it into the silo. In order to

prevent the silage from spoiling, five cows were used in each group fed silage, although there were only three animals in the other groups.

For the second year's work grade Jersey cows, comparable with those used in the first year's experiments, were purchased from the same source. The 18 cows used were grouped and fed as follows (Table II):

TABLE II.—*Grouping and rations of cows in second year's tests* [a]

Number of cows in group.	Ration.	
	First period (80 days).	Second period (80 days).
5............	Grain, cottonseed hulls, and medium prickly-pear.	Grain, cottonseed hulls, and sorghum silage.
5............	Grain, cottonseed hulls, and sorghum silage.	Grain, cottonseed hulls, and medium prickly-pear.
3............	Grain, cottonseed hulls, and medium prickly-pear.	Grain and cottonseed hulls.
3............	Grain and cottonseed hulls	Grain, cottonseed hulls, and medium prickly-pear.
1............	Cottonseed meal and prickly-pear *ad libitum*.	
1............	Prickly-pear alone.	

[a] There was a transition period of 10 days between the two 80-day periods.

Cow 5 in the first group, a heavy-producing cow that had a roughage ration of cottonseed hulls, became so ill that it was necessary to change the character of her ration at the end of 70 days in the first 80-day period. With this individual the second feeding period was cut from 80 to 70 days, so as to make a better comparison of the data obtained from the two periods. With all the other animals the data were collected for the full periods of 80 days each.

The digestible nutrients of the feeds in the first year's trials were estimated from actual digestion trials. (See p. 418.) As no digestion trials of the feed used during the second year's work were made, the coefficients of digestion used in calculating the nutrients digested were taken from Henry (8, pp. 572–577), except for prickly-pear, in which case our own figures were used. The energy values were calculated by the Armsby (1) method.

EXPERIMENTAL WORK

MILK PRODUCTION, FEEDS, AND BODY WEIGHTS

The principal data by groups, showing in comparative form the results of feeding the various rations, are given in Tables III to VI. Table III deals with milk production, including the fat and solid contents of the milk; Table IV shows the amount of each of the feeds consumed and the body weights; Table V, the nutrients digested; and Table VI, the energy values of the feed consumed. Complete data for the individual animals will be found in Tables XX to XLII.

TABLE III.—*Effect of different feeds on milk production*

FIRST YEAR

Group.	Nos. of cows.	80-day period.	Total milk.	Fat. Average per cent.	Fat. Total quantity.	Total solids.
			Pounds.	*Per cent.*	*Pounds.*	*Pounds.*
Heavy prickly-pear....................	1, 2, 3	First....	3,716.0	3.90	144.87	475.97
Medium prickly-pear...................	4, 5, 6	...do.....	4,676.6	4.42	206.53	630.82
Heavy prickly-pear....................	4, 5, 6	Second..	4,442.3	3.93	174.76	568.41
Medium prickly-pear...................	1, 2, 3	..do.....	3,206.8	4.14	132.91	416.06
Total for heavy prickly-pear groups.	1, 2, 3 / 4, 5, 6	First.... / Second..	8,158.3	3.92	319.63	1,044.38
Total for medium prickly-pear groups.	4, 5, 6 / 1, 2, 3	First.... / Second..	7,883.4	4.31	339.44	1,046.88
Medium prickly-pear....................	8, 9	First....	3,543.1	3.81	135.10	441.81
Sorghum hay...........................	10, 11, 12	...do.....	4,611.3	4.87	224.55	654.87
Medium prickly-pear...................	10, 11, 12	Second..	4,385.8	4.33	189.98	588.78
Sorghum hay	8, 9	..do.....	3,230.8	4.04	130.69	409.62
Total for medium prickly-pear groups.	8, 9 / 10, 11, 12	First.... / Second..	7,928.9	4.10	325.03	1,033.59
Total for hay groups.............	10, 11, 12 / 8, 9	First.... / Second..	7,842.1	4.53	355.24	1,064.49

SECOND YEAR

Group.	Nos. of cows.	80-day period.	Total milk.	Fat. Average per cent.	Fat. Total quantity.	Total solids.
Medium prickly-pear...................	6, 9, 15, 16, 18	First....	8,804.1	4.29	377.68	1,157.70
Sorghum silage	3, 14, 17, 19, 20	...do.....	7,638.5	5.14	392.86	1,103.93
Medium prickly-pear...................	3, 14, 17, 19, 20	Second..	6,817.6	4.74	323.41	975.87
Sorghum silage	6, 9, 15, 16, 18	...do.....	7,649.0	4.72	360.92	1,084.74
Total for medium prickly-pear groups.	6, 9, 15, 16, 18 / 3, 14, 17, 19, 20	First.... / Second..	15,621.7	4.48	701.09	2,133.57
Total for sorghum-silage groups ..	3, 14, 17, 19, 20 / 6, 9, 15, 16, 18	First.... / Second..	15,287.5	4.93	753.78	2,188.67
Medium prickly-pear...................	8, 11, 12	First....	6,354.4	4.56	289.80	875.26
Cottonseed hulls [a]...................	4, 5, 10	...do.....	6,075.2	4.90	298.00	857.44
Medium prickly-pear [a]...............	4, 5, 10	Second..	4,550.2	4.43	201.66	618.48
Cottonseed hulls......................	8, 11, 12	...do.....	5,189.8	4.97	258.20	737.39
Total for medium prickly-pear groups.[a]	8, 11, 12 / 4, 5, 10	First.... / Second..	10,904.6	4.51	491.46	1,493.74
Total for cottonseed-hulls groups..	4, 5, 10 / 8, 11, 12	First.... / Second..	11,265.0	4.94	556.20	1,594.83

[a] Cow 5: 70-day period.

TABLE IV.—*Effect of feeds consumed on body weights of dairy cows*

FIRST YEAR

Group.	Nos. of cows.	80-day period.	Grain.	Sorghum hay.	Cottonseed hulls.	Prickly-pear.	Sorghum silage.
			Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Heavy prickly-pear.......	1, 2, 3	First....	1,380.0	1,177.5	....	34,451	....
Medium prickly-pear.....	4, 5, 6	...do....	1,923.0	2,401.0	....	17,795	....
Heavy prickly-pear.......	4, 5, 6	Second..	1,790.0	995.5	....	27,248	....
Medium prickly-pear.....	1, 2, 3	...do....	1,452.6	2,113.5	....	14,400	....
Total for heavy prickly-pear groups...........	1, 2, 3 / 4, 5, 6	First.... / Second..	3,170.0	2,173.0	....	61,699	....
Total for medium prickly-pear groups.	4, 5, 6 / 1, 2, 3	First.... / Second..	3,375.6	4,514.5	....	32,195	....
Medium prickly-pear.....	8, 9	First....	1,288.0	1,321.5	....	11,880	....
Sorghum hay.............	10, 11, 12	...do....	2,088.6	4,165.5	....	....	....
Medium prickly-pear.....	10, 11, 12	Second..	1,888.9	2,049.5	....	14,400	....
Sorghum hay.............	8, 9	...do....	1,379.1	2,396.5	....	....	....
Total for medium prickly-pear groups.	8, 9 / 10, 11, 12	First.... / Second..	3,176.9	3,371.0	....	26,280	....
Total for hay groups	10, 11, 12 / 8, 9	First.... / Second..	3,467.7	6,562.0	....	....	....

TABLE IV.—*Effect of feeds consumed on body weights of dairy cows*—Continued

SECOND YEAR

Group.	Nos. of cows.	80-day period.	Grain.	Sorghum hay.	Cotton-seed hulls.	Prickly-pear.	Sorghum silage.
			Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Medium prickly-pear.....	6, 9, 15, 16, 18	First....	3, 811. 0	....	3, 886	29, 975	....
Sorghum silage..........	3, 14, 17, 19, 20	...do....	3, 894. 0	....	3, 874	....	10, 849. 5
Medium prickly-pear.....	3, 14, 17, 19, 20	Second..	3, 301. 9	....	3, 540	29, 240	....
Sorghum silage..........	6, 9, 15, 16, 18	...do....	3, 685. 1	....	3, 998	....	8, 499. 0
Total for medium prickly-pear groups.	6, 9, 15, 16, 18 / 3, 14, 17, 19, 20	First.... / Second..	7, 112. 9	....	7, 426	59, 215	....
Total for sorghum-silage groups......	3, 14, 17, 19, 20 / 6, 9, 15, 16, 18	First.... / Second..	7, 579. 1	....	7, 872	....	19, 348. 5
Medium prickly-pear.....	8, 11, 12	First....	3, 181. 0	....	2, 080	17, 080	....
Cottonseed hulls[a].......	4, 5, 10	...do....	3, 220. 7	....	4, 577	....	....
Medium prickly-pear[a]....	4, 5, 10	Second..	2, 086. 0	....	1, 884	16, 677	....
Cottonseed hulls..........	8, 11, 12	...do....	2, 683. 0	....	4, 244	....	....
Total for medium prickly-pear groups.[a]	8, 11, 12 / 4, 5, 10	First.... / Second..	5, 267. 0	....	3, 964	33, 757	....
Total for cottonseed-hulls groups[a].....	4, 5, 10 / 8, 11, 12	First.... / Second..	5, 903. 7	....	8, 821	....	....

FIRST YEAR

Group.	Nos. of cows.	80-day period.	Initial weight.	Final weight.	Gain.
			Pounds.	*Pounds.*	*Pounds.*
Heavy prickly-pear...................	1, 2, 3	First....	2, 263. 0	2, 327. 8	64. 8
Medium prickly-pear..................	4, 5, 6	...do....	2, 215. 2	2, 309. 4	94. 2
Heavy prickly-pear...................	4, 5, 6	Second..	2, 389. 2	2, 421. 6	32. 4
Medium prickly-pear..................	1, 2, 3	...do....	2, 216. 6	2, 213. 2	− 3. 4
Total for heavy prickly-pear groups	1, 2, 3 / 4, 5, 6	First.... / Second..	4, 652. 2	4, 749. 4	97. 2
Total for medium prickly-pear groups.	4, 5, 6 / 1, 2, 3	First.... / Second..	4, 431. 8	4, 522. 6	90. 8
Medium prickly-pear..................	8, 9	First....	1, 315. 5	1, 389. 8	74. 3
Sorghum hay..........................	10, 11, 12	...do....	1, 961. 1	2, 052. 6	91. 5
Medium prickly-pear..................	10, 11, 12	Second..	2, 079. 2	2, 101. 6	22. 4
Sorghum hay..........................	8, 9	...do....	1, 397. 2	1, 381. 2	−16. 0
Total for medium prickly-pear groups.	8, 9 / 10, 11, 12	First.... / Second..	3, 394. 7	3, 491. 4	96. 7
Total for hay groups...............	10, 11, 12 / 8, 9	First.... / Second..	3, 358. 3	3, 433. 8	75. 5

SECOND YEAR

Group.	Nos. of cows.	80-day period.	Initial weight.	Final weight.	Gain.
Medium prickly-pear..................	6, 9, 15, 16, 18	First....	3, 936. 2	4, 070. 8	134. 6
Sorghum silage.......................	3, 14, 17, 19, 20	...do....	3, 948. 2	4, 116. 0	167. 8
Medium prickly-pear..................	3, 14, 17, 19, 20	Second..	4, 259. 0	4, 262. 2	3. 2
Sorghum silage.......................	6, 9, 15, 16, 18	...do....	4, 028. 2	3, 995. 2	−33. 0
Total for medium prickly-pear groups.	6, 9, 15, 16, 18 / 3, 14, 17, 19, 20	First.... / Second..	8, 195. 2	8, 333. 0	137. 8
Total for sorghum-silage groups.....	3, 14, 17, 19, 20 / 6, 9, 15, 16, 18	First.... / Second..	7, 976. 4	8, 111. 2	134. 8
Medium prickly-pear..................	8, 11, 12	First....	2, 351. 0	2, 406. 0	55. 0
Cottonseed hulls[a]...................	4, 5, 10	...do....	2, 412. 0	2, 436. 6	24. 6
Medium prickly-pear[a]................	4, 5, 10	Second..	2, 429. 8	2, 469. 4	39. 6
Cottonseed hulls.....................	8, 11, 12	...do....	2, 349. 2	2, 419. 8	70. 6
Total for medium prickly-pear groups.[a]	8, 11, 12 / 4, 5, 10	First.... / Second..	4, 780. 8	4, 875. 4	94. 6
Total for cottonseed-hulls groups[a]..	4, 5, 10 / 8, 11, 12	First.... / Second..	4, 761. 2	4, 856. 4	95. 2

[a] Cow 5: 70-day period.

TABLE V.—*Amount of nutrients digested by dairy cows on different rations*

FIRST YEAR

Ration.	Nos. of cows.	80-day period.	Dry matter.	Protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
			Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Heavy prickly-pear.....	1, 2, 3	First....	3,155.85	609.78	506.66	1,543.49	109.60
Medium prickly-pear...	4, 5, 6	...do.....	3,171.38	530.83	607.80	1,666.07	145.05
Heavy prickly-pear.....	4, 5, 6	Second..	3,584.97	617.26	383.36	1,918.23	164.95
Medium prickly-pear...	1, 2, 3	...do.....	3,106.37	395.69	529.32	1,772.44	147.35
Total for heavy prickly-pear groups.	1, 2, 3 First.... / 4, 5, 6 Second.		6,740.82	1,227.04	890.02	3,461.72	274.55
Total for medium prickly-pear groups.	4, 5, 6 First.... / 1, 2, 3 Second.		6,277.75	926.52	1,137.12	3,438.51	292.40
Medium prickly-pear...	8, 9	First....	2,010.86	349.31	362.39	1,060.90	94.09
Sorghum hay..........	10, 11, 12	...do.....	3,047.02	511.84	731.23	1,541.28	161.24
Medium prickly-pear...	10, 11, 12	Second..	3,369.40	476.18	531.73	1,916.57	177.47
Sorghum hay..........	8, 9	...do.....	2,206.07	318.93	491.27	1,208.67	129.94
Total for medium prickly-pear groups.	10, 11, 12 Second.. / 8, 9 First....		5,380.26	825.49	894.12	2,977.47	271.56
Total for hay groups.	10, 11, 12 ...do..... / 8, 9 Second..		5,253.09	830.77	1,222.50	2,749.95	291.18

SECOND YEAR

Ration.	Nos. of cows.	80-day period.	Dry matter.	Protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
Medium prickly-pear...	6, 9, 15, 16, 18	First....	5,601.60	843.01	1,016.80	3,198.84	207.88
Sorghum silage.........	3, 14, 17, 19, 20	...do.....	5,806.49	882.53	1,313.82	3,019.28	260.27
Medium prickly-pear...	3, 14, 17, 19, 20	Second..	4,818.52	745.48	939.78	2,679.92	175.86
Sorghum silage.........	6, 9, 15, 16, 18	...do.....	5,317.34	825.97	1,222.94	2,734.64	242.29
Total for medium prickly-pear groups.	6, 9, 15, 16, 18 First.... / 3, 14, 17, 19, 20 Second..		10,420.12	1,588.49	1,956.58	5,878.76	383.74
Total for sorghum silage.	3, 14, 17, 19, 20 First.... / 6, 9, 15, 16, 18 Second..		11,123.83	1,708.50	2,536.76	5,753.92	502.56
Medium prickly-pear...	8, 11, 12	First....	3,867.15	684.32	568.62	2,251.82	159.68
Cottonseed hulls [a]......	4, 5, 10	...do.....	4,071.76	663.07	1,039.84	2,026.90	173.88
Medium prickly-pear [a]..	4, 5, 10	Second..	2,838.98	466.14	510.84	1,595.67	107.94
Cottonseed hulls........	8, 11, 12	...do.....	3,513.62	556.47	979.58	1,695.86	136.91
Total for medium prickly-pear groups.[a]	8, 11, 12 First.... / 4, 5, 10 Second..		6,706.13	1,150.46	1,079.46	3,847.49	267.62
Total for cotton-seed-hulls groups.[a]	4, 5, 10 First.... / 8, 11, 12 Second..		7,585.38	1,219.54	2,019.42	3,722.76	310.79

[a] Cow No. 5: 70-day period.

TABLE VI.—*Energy value of feed consumed by dairy cows on different rations*

FIRST YEAR

Group.	Nos. of cows.	80-day period.	Cottonseed meal.	Wheat bran.	Corn chop.	Prickly-pear.
			Therms.	*Therms.*	*Therms.*	*Therms.*
Heavy prickly-pear.............	1, 2, 3	First....	389.05	212.44	360.72	1,210.81
Medium prickly-pear...........	4, 5, 6	...do.....	512.17	302.18	532.95	666.94
Heavy prickly-pear.............	4, 5, 6	Second..	549.73	285.52	464.73	1,307.31
Medium prickly-pear...........	1, 2, 3	...do.....	427.28	236.74	400.00	740.05
Total for heavy prickly-pear groups.	1, 2, 3 First.... / 4, 5, 6 Second..		938.78	497.96	825.45	2,518.12
Total for medium prickly-pear groups.	4, 5, 6 First.... / 1, 2, 3 Second..		939.45	538.92	932.95	1,406.99
Medium prickly-pear...........	8, 9	First....	343.02	202.36	356.92	445.28
Sorghum hay..................	10, 11, 12	...do.....	569.62	328.72	572.47	....
Medium prickly-pear...........	10, 11, 12	Second..	555.59	307.83	520.15	740.05
Sorghum hay..................	8, 9	...do.....	414.75	225.12	375.78	....
Total for medium prickly-pear groups.	8, 9 First.... / 10, 11, 12 Second..		898.61	510.19	877.07	1,185.33
Total for hay groups......	10, 11, 12 First.... / 8, 9 Second..		984.37	553.84	948.25	....

TABLE VI.—*Energy value of feed consumed by dairy cows on different rations*—Continued

SECOND YEAR

Group.	Nos. of cows.	80-day period.	Cotton-seed meal.	Wheat bran.	Corn chop.	Prickly-pear.
			Therms.	*Therms.*	*Therms.*	*Therms.*
Medium prickly-pear............	6,9,15,16,18	First....	995.21	635.84	1,129.07	1,191.55
Sorghum silage.................	3,14,17,19,20	...do.....	1,016.90	649.72	1,153.69	....
Medium prickly-pear............	3,14,17,19,20	Second..	809.39	550.02	962.45	980.46
Sorghum silage.................	6,9,15,16,18	...do.....	903.45	613.87	1,074.20	....
Total for medium prickly-pear groups.	6,9,15,16,18 / 3,14,17,19,20	First.... / Second..	}1,804.60	1,185.86	2,091.52	2,172.01
Total for sorghum silage groups.	6,9,15,16,18 / 3,14,17,19,20	...do..... / First....	}1,920.35	1,263.59	2,227.89	....
Medium prickly-pear............	8,11,12	...do.....	830.68	530.74	942.41	678.94
Cottonseed hulls [a]...............	4,5,10	...do.....	841.11	537.39	954.24	....
Medium prickly-pear [a]..........	4,5,10	Second..	511.35	347.47	608.02	559.18
Cottonseed hulls................	8,11,12	...do.....	657.72	446.92	782.03	....
Total for medium prickly-pear groups.[a]	8,11,12 / 4,5,10	First.... / Second..	}1,342.03	878.21	1,550.43	1,238.12
Total for cottonseed-hulls groups.[a]	4,5,10 / 8,11,12	First.... / Second..	}1,498.83	984.31	1,736.27	....

FIRST YEAR

Group.	Nos. of cows.	80-day period.	Sorghum silage.	Sorghum hay.	Cotton-seed hulls.	Total energy.
			Therms.	*Therms.*	*Therms.*	*Therms.*
Heavy prickly-pear.............	1,2,3	First....	....	264.17	....	2,437.19
Medium prickly-pear............	4,5,6	...do.....	....	613.10	....	2,627.34
Heavy prickly-pear.............	4,5,6	Second..	....	337.61	....	2,944.90
Medium prickly-pear............	1,2,3	...do.....	....	811.03	....	2,615.10
Total for heavy prickly-pear groups.	1,2,3 / 4,5,6	First.... / Second..	....	601.78	....	5,382.09
Total for medium prickly-pear groups.	4,5,6 / 1,2,3	First.... / Second..	....	1,424.13	....	5,242.44
Medium prickly-pear............	8,9	First....	....	337.44	....	1,685.92
Sorghum hay....................	10,11,12	...do.....	....	1,072.17	....	2,542.98
Medium prickly-pear............	10,11,12	Second..	....	787.49	....	2,911.11
Sorghum hay....................	8,9	...do.....	....	920.35	....	1,936.00
Total for medium prickly-pear groups.	8,9 / 10,11,12	First.... / Second..	....	1,124.93	....	4,596.13
Total for hay groups......	10,11,12 / 8,9	First.... / Second..	....	1,992.52	....	4,478.98

SECOND YEAR

Group.	Nos. of cows.	80-day period.	Sorghum silage.	Sorghum hay.	Cotton-seed hulls.	Total energy.
Medium prickly-pear............	6,9,15,16,18	First....	....	....	422.28	4,373.95
Sorghum silage.................	3,14,17,19,20	...do.....	1,180.51	....	424.48	4,425.30
Medium prickly-pear............	3,14,17,19,20	Second..	....	....	365.14	3,667.46
Sorghum silage.................	6,9,15,16,18	...do.....	965.52	....	412.36	3,969.40
Total for medium prickly-pear groups.	6,9,15,16,18 / 3,14,17,19,20	First.... / Second..	....	....	787.42	8,041.41
Total for sorghum silage groups.	6,9,15,16,18 / 3,14,17,19,20	...do..... / First....	}2,146.03	....	836.84	8,394.70
Medium prickly-pear............	8,11,12	...do.....	....	....	227.92	3,210.69
Cottonseed hulls [a]...............	4,5,10	...do.....	....	....	501.55	2,834.29
Medium prickly-pear [a]..........	4,5,10	Second..	....	....	194.33	2,220.35
Cottonseed hulls................	8,11,12	...do.....	....	....	437.78	2,324.45
Total for medium prickly-pear groups.[a]	8,11,12 / 4,5,10	First.... / Second..	....	....	422.25	5,431.04
Total for cottonseed-hulls groups.[a]	4,5,10 / 8,11,12	First.... / Second..	....	....	939.33	5,158.74

[a] Cow No. 5, 70-day period.

Tables III to VI show in general that prickly-pear produced more milk with a smaller percentage of fat than the other feeds with which it was compared. The total production of milk fat was reduced appreciably by feeding prickly-pear, while the production of milk solids was lowered but slightly.

In comparing the results of the first year's work, it will be noticed that the heavy prickly-pear ration produced more milk and less fat but the same amount of total solids as the medium prickly-pear ration. It will be seen also that the dry matter digested and the energy values of the feed were greater in the case of the heavy ration.

It will be observed that the medium prickly-pear ration produced slightly more milk but less fat and total solids than the sorghum-hay group. The cows also consumed more digestible nutrients, and the energy values were greater. While the body weights were not so well controlled as with the preceding groups, it is thought that the error on this account is of no great consequence.

Cows fed with prickly-pear produced a little more milk but less fat and less other solids than those fed on the sorghum silage. The cows of the sorghum-silage groups ate more digestible dry matter, and the energy value of their feed was greater.

The results in the test of medium rations of prickly-pear as compared with cottonseed hulls are somewhat different from the preceding. The cottonseed hulls not only produced more fat but also more milk and total solids than prickly-pear. There was more digestible dry matter in the ration of the cottonseed-hulls groups, but a smaller energy value.

The results as a whole seem to show that a moderate ration of prickly-pear was used more efficiently than a heavy ration. As will be seen later, the digestion of dry matter is lower for the heavy ration, and possibly the cows receiving a large amount of prickly-pear required more food for maintenance, especially in cold weather.

Ordinarily it would be better to feed a medium rather than a heavy ration of prickly-pear to milking cows because of the greater fat production and the more sanitary condition of the cows and stable. Prickly-pear in large quantities loosens the bowels, which makes it difficult to keep the cows clean. There are many cases, however, in which a large amount of these cacti might well be fed. If the product is to be disposed of as milk rather than as butter or cream and if supplementary roughages are relatively high in cost, it would be to the advantage of the dairyman to feed a large quantity of prickly-pear, provided extra precautions are taken to keep the cows and the stable clean. The proper amount to feed depends to some extent upon the ability of the individual animal to consume large quantities. It is thought that most cows will refuse to eat more than 100 pounds each day when supplemented with grain and a small amount of hay.

RELATIVE NUTRITIVE VALUE OF THE FEEDS

The following calculations show the method employed in estimating the relative nutritive values. To obtain the number of pounds of feed required to produce one pound of fat, the total number of pounds of feed was divided by the total fat production. The quantity of prickly-pear used to replace the hay or other feed was considered equal in value for fat production to the amount of feed which it replaced. The same method was followed in estimating the relative values of the different feeds on the bases of nutrients digested and energy values. The results are given in Table VII.

Table VIII contains different methods for comparing the relative nutritive value of the feeds tested, estimated from their chemical composition and digestibility. If the digestible nutrients or therms furnished an accurate means of estimating the relative values of different kinds of feeds, 1 pound of digestible nutrients or 1 therm in one kind of feed would be equivalent in producing power to 1 pound of digestible nutrients or 1 therm in another kind of feed. Thus, while 1.40 pounds of digestible nutrients in prickly-pear are equivalent to 1 pound of digestible nutrients in sorghum hay, 0.90 of a pound in the prickly-pear is equal to 1 pound in the cottonseed hulls, and while 1.61 therms in the prickly-pear are equal to 1 therm in the hay, 3.03 therms are required to equal 1 therm in cottonseed hulls. The digestible-nutrients method shows less variation than the energy-value method and for this reason is more accurate, in this investigation at least, but both show such wide variations as to make them of doubtful value in estimating the nutritive values.

On account of the variation in the water content of prickly-pear and sorghum hay, it was thought best to reduce them all to a dry-matter content that would be near the average for the particular feed. The relative values were estimated by using the following percentages of dry matter for the various feeds: Prickly-pear, 10 per cent; sorghum hay, 80 per cent; cottonseed hulls, 90 per cent; silage, 25 per cent. Calculated in this way, the relative values on the basis of feed consumed are as follows:

Medium prickly-pear and sorghum hay versus heavy prickly-pear and sorghum hay .... 1 pound of sorghum hay equals 15.9 pounds of prickly-pear.

Medium rations of prickly-pear versus sorghum hay............ 1 pound of sorghum hay equals 10.1 pounds of prickly-pear.

Medium rations of prickly-pear versus sorghum silage........... 1 pound of sorghum silage equals 2.6 pounds of prickly-pear.

Medium rations of prickly-pear versus cottonseed hulls .... 1 pound of cottonseed hulls equals 5.8 pounds of prickly-pear.

The prickly-pear used in these experiments contained perhaps more water than the average. Henry (8, p. 572-577) gives the water content of prickly-pear as 84.2 per cent, and Hare's (7) average of several analyses of a certain variety shows these cacti to have 83.41 per cent of water. Prickly-pear containing these percentages of water would be worth probably 50 or 60 per cent more than is last indicated.

TABLE VII.—*Relative values of sorghum hay and prickly-pear when the latter is fed in medium quantities*

ON BASIS OF FEED CONSUMED

Group.	Fat produced.	Total feed consumed.			Feed required for 1 pound of fat.		
		Grain.	Sorghum hay.	Prickly-pear.	Grain.	Sorghum hay.	Prickly-pear.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Medium prickly-pear..........	325.08	3,176.9	3,371.0	26,280.0	9.8	10.37	80.84
Sorghum hay [a]..............	355.24	3,467.7	6,562.0	....	9.8	18.47	....

ON BASIS OF NUTRIENTS DIGESTED

Group.	Fat produced.	Total nutrients digested.			Digestible nutrients required for 1 pound of fat.		
		Grain.	Sorghum hay.	Prickly-pear.	Grain.	Sorghum hay.	Prickly-pear.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Medium prickly-pear..........	325.08	2,101.70	1,543.75	1,323.19	6.5	4.75	4.07
Sorghum hay [b]..............	355.24	2,300.84	2,793.56	....	6.5	7.86	....

ON BASIS OF ENERGY VALUE

Group.	Fat produced.	Total energy in nutrients digested.			Energy required for 1 pound of fat.		
		Grain.	Sorghum hay.	Prickly-pear.	Grain.	Sorghum hay.	Prickly-pear.
	Pounds.	*Therms.*	*Therms.*	*Therms.*	*Therms.*	*Therms.*	*Therms.*
Medium prickly-pear..........	325.08	2,285.87	1,124.93	1,185.33	7.0	3.46	3.65
Sorghum hay [c]..............	355.24	2,486.46	1,992.52	....	7.0	5.61	....

[a] Quantity of hay required to replace 80.84 pounds of prickly-pear=18.47−10.37=8.10 pounds.
One pound of sorghum hay equals 10 pounds of prickly-pear.
[b] Quantity of digestible nutrients in hay required to replace 4.07 pounds digestible nutrients in prickly-pear is 7.86−4.75=3.11 pounds.
One pound of digestible nutrients in the hay equals 1.31 pounds digestible nutrients in prickly-pear.
[c] One therm in the hay equals 1.70 therms in the prickly-pear.

TABLE VIII.—*Relative values of prickly-pear and other feeds*

Basis of comparison.	Medium prickly-pear and sorghum hay v. heavy prickly-pear and hay.		Medium prickly-pear v. sorghum hay.		Medium prickly-pear v. sorghum silage.		Medium prickly-pear v. cottonseed hulls.	
	Sorghum hay.	Prickly-pear.	Sorghum hay.	Prickly-pear.	Sorghum silage.	Prickly-pear.	Cottonseed hulls.	Prickly-pear.
Feed consumed, pounds...........	1	15.1	1	10.0	1	3.3	1	8.8
Nutrients digested, pounds...........	1	1.4	1	1.31	1	1.02	1	.90
Energy values, therms............	1	1.61	1	1.70	1	1.09	1	3.03

DIGESTION TRIALS

Toward the close of the first year's work it became apparent that the heavy prickly-pear ration was not used as efficiently as the medium. In order to ascertain whether the heavy ration was rushed through the digestive tract too rapidly to allow sufficient time for digestion and absorption, and also to check previous work of that kind which had not been altogether satisfactory, some digestion trials were conducted in May and June, using cows that were accustomed to the different classes of roughage fed during the previous year's work.

The plan was to use two cows in each trial and to conduct five trials for a period of 10 days each, with a preliminary period of 4 to 7 days during which time the ration was to be exactly the same in kind and quantity as during the digestion trial. This plan was rigidly adhered to, except that in one of the trials data for 9 days instead of 10 were used.

The four different rations used were as follows: Sorghum hay and grain; sorghum hay, medium quantities of prickly-pear, and grain; sorghum hay, heavy rations of prickly-pear, and grain; and prickly-pear alone. The grain in every case consisted of equal parts by weight of corn meal, cottonseed meal, and wheat bran.

The sorghum hay was run through a cutter, thoroughly mixed, and a sample taken for analysis. Samples of the corn meal and other grains were taken before the grain mixture was prepared. The prickly-pear was sampled by taking a representative portion each day, chopping it into fine pieces, mixing and weighing out about 100 gm. on a chemical balance. This portion was then dried on a hot-water bath. The dried samples for 10 days were placed together in a tight jar and sent to Washington for analysis with the other samples.

The feces were collected by attendants who were with the cows day and night throughout the trials. No urine was collected. The cows were kept in ordinary rigid stanchions. The wooden gutter behind and the rear portion of the platform on which the cows stood were covered with white oilcloth, so that in case the attendant failed to catch the feces at the time they were passed, they could be easily collected from the cloth. In order to avoid any possibility of including urine with the feces, water was used to wash it out of the gutter. An ordinary shovel was used in catching the feces, except in the case of the cows receiving prickly-pear alone, when, owing to the extreme looseness of the bowels, it became necessary to use a large tub. The feces were placed in large cans, weighed each day at the same hour, and, after being thoroughly mixed, an aliquot portion was taken and composited for chemical analysis. These samples were preserved with chloroform and kept on ice. At the end of 10 days a portion of the composite was weighed on the chemical balances and then dried over the hot-water bath in the same way as the prickly-pear. Owing to the loss in nitrogen when feces are dried, the samples for nitrogen deter-

mination were taken from the fresh material, preserved with chemically pure sulphuric acid, and sent to the Washington laboratories for analysis.

Table IX gives coefficients of digestion for each animal in the different trials, together with the character of the ration and the amounts of each feed fed. Complete data for each individual animal will be found in Tables XX to XLII.

TABLE IX.—*Summary of coefficients of digestion for dairy cows*

No. of cow.	Ration.			Dry matter.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.	Organic matter.
	Grain.	Sorghum hay.	Prickly-pear.							
	Lbs.	Lbs.	Lbs.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
11	66	180	....	61.75	23.97	64.60	65.77	64.35	76.81	65.31
12	90	160	....	63.15	25.72	76.34	66.59	63.34	71.97	66.46
Average	....	....	....	62.45	24.84	70.47	66.18	63.84	74.39	65.88
11	66	100	600	63.79	25.17	71.05	64.25	69.19	70.18	68.48
12	90	100	600	64.70	28.76	74.21	62.22	68.69	84.45	69.07
1	42	100	600	58.46	26.15	63.00	60.49	63.62	64.15	62.87
Average	....	....	....	62.32	26.69	69.42	62.32	67.17	72.93	66.81
11	66	50	1,050	61.08	31.71	89.72	52.86	63.55	75.02	65.72
2	54	45	1,080	60.17	39.14	84.28	53.47	61.74	67.99	63.71
1	42	50	1,200	59.79	32.68	84.54	56.45	63.33	62.99	64.18
Average	....	....	....	60.35	34.51	86.18	54.26	62.87	68.67	64.54
1	....	....	1,170	58.20	36.87	75.29	38.09	66.93	76.93	63.84
3	....	....	1,200	64.96	39.88	67.83	47.88	76.17	54.83	70.59
Average	....	....	....	61.58	38.37	71.56	42.98	71.55	65.88	67.21

METHOD OF CALCULATING DIGESTIBLE NUTRIENTS

Table X gives the estimated coefficients of digestion for each class of rations fed during the digestion trials, the actual coefficients of digestion as determined by these trials, and the factor showing the difference between the estimated and the actual coefficients. In arriving at the estimated coefficients the average figures for sorghum fodder, corn, wheat bran, and cottonseed meal were taken from Henry (8, p. 572–577), while the figures for prickly-pear were taken from our own results as given in Table IX. In determining the digestible nutrients consumed by the different groups of cows in this feeding experiment the crude nutrients consumed were multiplied by the average coefficients of digestion as taken from Henry and the resulting products multiplied by the factor of difference for that particular group. This method is given with a full knowledge of the error involved in assuming that the coefficients of digestion for the different constituents are increased or decreased in the same ratio for each ingredient of the ration. As a matter of fact, it is probable that in some rations the digestibility of one ingredient may be reduced while that of another may be increased. However, calculations using average coefficients and the factors of difference are thought to be more nearly repre-

sentative of actual conditions than calculations involving only the use of the average coefficients.

TABLE X.—*Comparison of estimated and actual coefficients of digestion for dairy cattle*

HAY GROUP

Coefficient of digestion.	Nos. of cows.	Dry matter.	Crude protein.	Crude fiber.	Nitro-gen-free extract.	Ether extract.
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Estimated	11, 12	64. 56	68. 20	48. 19	68. 46	78. 46
Actual	11, 12	62. 45	70. 47	66. 18	63. 84	74. 21
Factor of difference		. 97	1. 03	1. 37	. 93	. 95

MEDIUM PRICKLY-PEAR GROUP

Estimated	1, 11, 12	64. 84	70. 91	46. 99	70. 64	77. 82
Actual	1, 11, 12	62. 32	69. 42	62. 32	67. 17	72. 93
Factor of difference		. 96	. 98	1. 33	. 95	. 94

HEAVY PRICKLY-PEAR GROUP

Estimated	1, 2, 11	64. 01	73. 08	45. 55	72. 03	76. 68
Actual	1, 2, 11	60. 35	86. 18	54. 26	62. 87	68. 67
Factor of difference		. 94	1. 18	1. 19	. 87	. 90

INFLUENCE OF PRICKLY-PEAR ON DIGESTIBILITY OF OTHER FEEDS

It has been claimed that prickly-pear is more valuable than its analysis indicates, for the reason that it increases the digestibility of any material with which it may be fed. To determine the accuracy of this statement Table XI was prepared by applying average coefficients of digestion to the nutrients fed in the digestion trials as determined from the weight and analyses of the feeds. These were then compared with the actual coefficients.

TABLE XI.—*Comparison of estimated and actual coefficients of digestion for dairy cows*

COMPARISON OF HAY, GRAIN, AND MEDIUM RATIONS OF PRICKLY-PEAR

Coefficient of digestion.	Nos. of cows.	Dry matter.	Crude protein.	Crude fiber.	Nitro-gen-free extract.	Ether extract.	Organic matter.
Hay and grain:		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Estimated	11, 12	64. 56	68. 20	48. 19	68. 46	78. 49	63. 80
Actual	11, 12	62. 45	70. 47	66. 18	63. 84	74. 21	65. 88
Difference							+2. 08
Medium prickly-pear, hay, and grain:							
Estimated	11, 12	65. 70	72. 47	46. 76	71. 39	79. 17	66. 92
Actual	11, 12	64. 24	72. 63	63. 23	68. 94	77. 31	68. 77
Difference							+1. 85

TABLE XI.—*Comparison of estimated and actual coefficients of digestion for dairy cows*—Continued

COMPARISON OF HAY, GRAIN, AND MEDIUM AND HEAVY RATIONS OF PRICKLY-PEAR

Cofficient of digestion.	Nos. of cows.	Dry matter.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.	Organic matter.
Medium prickly-pear, hay, and grain:		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Estimated.................	11, 1	64.01	69.48	47.16	69.86	76.88	65.12
Actual.....................	11, 1	61.12	67.02	62.37	66.40	67.16	65.67
Difference.............							+0.55
Heavy prickly-pear, hay, and grain:							
Estimated.................	11, 1	63.54	72.90	45.55	71.94	76.73	65.57
Actual.....................	11, 1	60.43	87.13	54.65	63.44	69.00	64.95
Difference.............							—2.62
Prickly-pear alone:							
Actual.....................	1, 3	61.58	71.56	42.98	71.55	65.88	67.21

It will be observed from this table that when hay and grain were fed the actual coefficient for organic matter exceeded the estimated by 2.08 per cent, and that when hay, grain, and a moderate quantity of prickly-pear (60 pounds per day) were fed the actual exceeded the estimated by 1.85 per cent. These results indicate that prickly-pear in moderate amounts has little effect on the digestibility of the other organic ingredients of the ration. The effect of a large ration of prickly-pear (105 to 120 pounds) as compared with a medium ration will be seen from the results with cows 11 and 1. When fed the medium ration, the actual exceeded the estimated coefficient by 0.55 per cent; when fed the large ration, the actual was 2.62 per cent less than the estimate. These figures indicate that a large amount of prickly-pear in the ration depressed the coefficient of digestion.

The results of the writers' digestion trials with prickly-pear as the sole feed agree fairly well with those obtained by Hare (7). The trials reported in this paper show a less efficient digestion of dry matter, fiber, nitrogen-free extract, ether extract, and organic matter, but a more efficient digestion of ash and especially of protein. In general, the coefficients of digestion secured by Hare are greater than those obtained in the trials of the writers (Table XII).

TABLE XII.—*Comparison of digestion coefficients of prickly-pear with those of previous trials*

Ration entirely of prickly-pear.	Dry matter.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.	Organic matter.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Average of our trials (see Table IX).................	61.58	38.37	71.56	42.98	71.55	65.88	67.21
Average of Hare's trials.........	64.91	35.69	49.56	47.66	80.77	68.46	72.76
Difference.................	—3.33	+2.68	+22.00	—4.68	—9.22	—2.58	—5.55

INFLUENCE OF PRICKLY-PEAR ON PERCENTAGE OF FAT IN MILK

It is a common belief of many farmers that the percentage of fat in milk varies according to the character of the ration; on the other hand, the results from several experiment stations indicate that the fat content is an individual characteristic that is not affected by the feed. The average percentage of fat in the milk for each cow during the entire feeding periods is given in Table XIII and shows conclusively that prickly-pear in the ration caused a reduction in the percentage of fat.

TABLE XIII.—*Influence of prickly-pear on percentage of fat in milk. Averages for the various feeds during full periods of 80 days*

No. of cow.	80-day period.	Feed.	Fat.	80-day period.	Feed.	Fat.
			Per cent.			*Per ct.*
1...............	First....	Heavy prickly-pear...	3.88	Second..	Medium prickly-pear..	3.87
2...............	...do.....	.....do...............	3.83	...do.....	.....do...............	4.09
3...............	...do.....	.....do...............	3.98	...do.....	.....do...............	4.34
4...............	Second..	.....do...............	4.35	First....	.....do...............	4.92
5...............	...do.....	.....do...............	3.76	...do.....	.....do...............	4.15
6...............	...do.....	.....do...............	3.86	...do.....	.....do...............	4.32
Average...			3.94			4.28
8...............	First....	Medium prickly-pear..	3.91	Second..	Sorghum hay.........	4.11
9...............	...do.....	.....do...............	3.68	...do.....	.....do...............	3.97
10..............	Second..	.....do...............	3.78	First....	.....do...............	4.24
11..............	...do.....	.....do...............	3.77	...do.....	.....do...............	4.52
12..............	...do.....	.....do...............	5.37	...do.....	.....do...............	5.88
Average...			4.10			4.54
4...............	Second..	Medium prickly-pear..	4.75	First....	Cottonseed hulls......	5.18
5...............	...do.....	.....do...............	4.42	...do.....	.....do...............	5.03
10..............	...do.....	.....do...............	4.11	...do.....	.....do...............	4.44
8...............	First....	.....do...............	3.95	Second..	.....do...............	4.23
11..............	...do.....	.....do...............	4.17	...do.....	.....do...............	4.46
12..............	...do.....	.....do...............	5.72	...do.....	.....do...............	6.19
Average...			4.52			4.92
3...............	Second..	Medium prickly-pear..	4.12	First....	Sorghum silage........	4.49
14..............	...do.....	.....do...............	4.74	...do.....	.....do...............	5.00
17..............	...do.....	.....do...............	4.79	...do.....	.....do...............	5.13
19..............	...do.....	.....do...............	5.26	...do.....	.....do...............	5.60
20..............	...do.....	.....do...............	4.92	...do.....	.....do...............	5.51
6...............	First....	.....do...............	4.39	Second..	.....do...............	5.02
9...............	...do.....	.....do...............	3.96	...do.....	.....do...............	4.08
15..............	...do.....	.....do...............	4.12	...do.....	.....do...............	4.42
16..............	...do.....	.....do...............	4.03	...do.....	.....do...............	4.46
18..............	...do.....	.....do...............	5.16	...do.....	.....do...............	6.01
Average...			4.55			4.97

In order to ascertain the more immediate effect on the fat percentage of feeding prickly-pear, Table XIV has been prepared, showing a comparison between the last 10-day period preceding the change in the ration and the first 10-day period after the change. As stated before, 10 days were allowed between the periods for making the change.

TABLE XIV.—*Influence of prickly-pear on percentage of fat in milk. Averages for the various feeds for 10 days before and 10 days after the change of ration*

No. of cow.	10-day period.	Feed.	Fat.	10-day period.	Feed.	Fat.
			Per ct.			*Per ct.*
1...............	Before change.	Heavy prickly-pear.	3. 70	After change..	Medium prickly-pear.	3. 80
2................	.....do........	.....do.........	3. 75	.....do........	.....do...........	3. 90
3................	.....do........	.....do.........	3. 85	.....do........	.....do...........	4. 05
4................	After change..	.....do.........	4. 30	Before change.	.....do...........	4. 60
5................	.....do........	.....do.........	3. 75	.....do........	.....do...........	4. 15
6................	.....do........	.....do.........	3. 85	.....do........	.....do...........	4. 00
Average...			3. 87			4. 08
8................	Before change.	Medium prickly-pear.	3. 90	After change..	Sorghum hay.....	4. 60
9................	.....do........	.....do.........	3. 50	.....do........	.....do...........	3. 90
10...............	After change..	.....do.........	3. 70	Before change.	.....do...........	4. 20
11...............	.....do........	.....do.........	3. 85	.....do........	.....do...........	4. 40
12...............	.....do........	.....do.........	5. 55	.....do........	.....do...........	6. 20
Average...			4. 10			4. 66
4...............	After change..	Medium prickly-pear.	4. 80	Before change.	Cottonseed hulls..	5. 10
5................	.....do........	.....do.........	4. 20	.....do........	.....do.........	5. 20
10...............	.....do........	.....do.........	4. 40	.....do........	.....do.........	4. 25
8................	Before change.	.....do.........	3. 75	After change..	.....do.........	3. 95
11...............	.....do........	.....do.........	4. 00	.....do........	.....do.........	4. 45
12...............	.....do........	.....do.........	5. 60	.....do........	.....do.........	6. 00
Average...			4. 46			4. 82
3...............	After change..	Medium prickly-pear.	4. 35	Before change.	Sorghum silage....	4. 75.
14...............	.....do........	.....do.........	4. 80	.....do........	.....do.........	5. 30
17...............	.....do........	.....do.........	5. 00	.....do........	.....do.........	5. 55
19...............	.....do........	.....do.........	5. 35	.....do........	.....do.........	5. 80
20...............	.....do........	.....do.........	5. 20	.....do........	.....do.........	5. 80
6...............	Before change.	.....do.........	4. 30	After change..	.....do.........	4. 90
9................	.....do........	.....do.........	3. 80	.....do........	.....do.........	4. 00
15...............	.....do........	.....do.........	4. 15	.....do........	.....do.........	4. 35
16...............	.....do........	.....do.........	4. 00	.....do........	.....do.........	4. 40
18...............	.....do........	.....do.........	5. 25	.....do........	.....do.........	5. 70
Average...			4. 62			5. 05

INFLUENCE OF PRICKLY-PEAR ON FLAVOR OF MILK AND QUALITY OF BUTTER

The milk from the experimental cows was tested for flavor almost daily throughout the feeding trials, and at no time was there any objectionable flavor detected. Some dairymen in southern Texas believe that prickly-pear injures the keeping quality of milk. In order to ascertain whether there was any basis for this belief, samples were on three different occasions taken from cows that were receiving prickly-pear and from cows that were fed hay as the only roughage. These samples were placed in clean bottles and kept at ordinary atmospheric temperature. No difference was observed in the time required for souring, and no objectionable flavor was manifest at any time. It is probable that under ordinary farm conditions milk from cows fed on prickly-pear does not keep so well as milk from cows fed on hay, because milk from the former is more liable to become contaminated with acid-producing bacteria, owing to the laxative character of prickly-pear and the consequent insanitary condition of the cow and stable.

Two tests were made to ascertain the effect of prickly-pear upon the quality of butter. No expert judges were available for scoring the butter, but the tests indicate that prickly-pear exerts little, if any, unfavorable influence on the flavor. Some difficulty was experienced in churning the cream of the cow fed on cottonseed meal, with prickly-pear as the sole roughage. Before butter could be made from this lot of cream it was necessary to raise it to a temperature of 65° F., five degrees higher than the churning temperature of the other lots of cream. Both the difficult churning of the cream and the hardness of this lot of butter seemed to be due to the cottonseed meal. The only noticeable effect of prickly-pear was the higher color of the butter (Table XV).

TABLE XV.—*The influence of prickly-pear upon the quality of butter*

No. of cow.	Feed.	Temperature of cream.	Length of churning period.	Color of butter.	Flavor of butter.	Hardness of butter.
		° F.	Minutes.			
12....	Mixed grain, hay..................	58	10	White.....	Good......	Medium.
13....	Cottonseed meal, prickly-pear......	65	20	Yellow....	Good......	Hard.
5.....	Mixed grain, hay, prickly-pear.....	60	14	Yellow....	Good......	Medium.
8.....	Grain, hay.........................	60	12	White.....	Good......	Medium.

EFFECT OF FEEDING PRICKLY-PEAR ALONE AS ROUGHAGE

Cow 13 was placed on a daily ration of about 4 pounds of cottonseed meal and as much prickly-pear as she would eat. This ration was continued for 371 days. During this time she consumed a total of 1,428 pounds of cottonseed meal, an average of 3.85 pounds a day, and 41,730 pounds of prickly-pear, an average of 112.5 pounds a day. At the beginning of this period the cow weighed 737 pounds, at the close 696 pounds, a loss of 41 pounds. During this time she produced 1,045 pounds of milk, containing 54.5 pounds of fat, and gave birth to a calf. Soon after being placed on this ration containing only prickly-pear as roughage her coat became rough, she scoured badly, and gave other evidence that she was not properly nourished. After 371 days of feeding on prickly-pear and cottonseed meal, the latter was withdrawn from the ration and prickly-pear alone was continued for 80 days. During this period she consumed a total of 9,919 pounds of prickly-pear, an average of 124 pounds a day, and produced but 24 pounds of milk and 1.1 pounds of fat, as her milk production was very low and she was dried up within 10 days after the beginning of the period. During this 80-day period she lost 24 pounds in weight. A ration of mixed grain, sorghum hay, and prickly-pear was then given to her. Within a week she stopped scouring and improved rapidly in appearance and condition. Apparently there were no permanent ill effects from her extended ration of prickly-pear.

Cow 1 was fed an average daily ration of 5 pounds of cottonseed meal and 141.7 pounds of prickly-pear for a period of 170 days. This cow

weighed 850 pounds at the beginning and 851 pounds at the close. She produced 2,204.8 pounds of milk and 87.76 pounds of fat. During the preceding year, under similar conditions except for feed, she produced in the same length of time 1,522.4 pounds of milk and 58.98 pounds of fat. Her feed during the first year consisted of mixed grain, hay, and prickly-pear. Owing to better physical condition at time of parturition, all the cows used for the first year's work gave an increase of both milk and butter fat during the second year's work. However, the percentage of increase of cow 1 was double that of the average of the remaining cows. This cow remained in good condition all the time and appeared to be as well nourished as the preceding year. Like cow 13, she scoured badly, but ceased to do so when the character of the ration was changed.

It is evident that there is a great difference in individual cows in their ability to subsist upon a ration containing prickly-pear as the sole roughage. Cow 1 always ate prickly-pear with great relish; cow 13 always ate it reluctantly. In fact, these two cows perhaps represented the two extremes as regards their appetite for prickly-pear. It is evident that the matter of palatability is an important consideration in determining whether or not to feed a ration containing prickly-pear as the sole roughage.

Summing up the results obtained with these two cows, it was found that both scoured badly but that neither was permanently injured in any way. One of them thrived, while the other did not. The explanation offered for this is the difference in the individuality of the two cows.

It may not be wise, however, to feed any cows exclusively on prickly-pear, as it may cause an obstruction of the intestine and the death of the animal. Cow 2, which was fed 120 pounds of prickly-pear each day, became ill following the experimental work, and after seven days of feeding on prickly-pear alone refused to eat feed of any kind. Prior to being fed on prickly-pear alone this cow was on a ration of 120 pounds of prickly-pear, 5 pounds of sorghum hay, and 6 pounds of mixed grain a day for a period of 15 days. Her illness was diagnosed as an obstruction of the intestine, and every effort was made to remove it. When a post-mortem examination was made, a tightly compressed mass of fiber about the size of a goose egg was found at the beginning of the small intestine. In addition, there was a large amount of undigested fiber closely matted together in the fourth stomach. No other animals suffered from any trouble of this sort, although three other cows were fed a ration of prickly-pear only, and at different times during these experiments seven were fed a heavy prickly-pear ration.

INFLUENCE OF THE MINERAL MATTER CONTAINED IN PRICKLY-PEAR

Prickly-pear contains a large amount of mineral matter, which no doubt is responsible in part for the well-known laxative nature of the plant. While in ordinary feed practice this high content of mineral matter is perhaps of no advantage, it may be desirable if the remainder

of the ration is deficient in mineral matter. Cows producing large quantities of milk require considerable mineral matter, especially calcium; and as some feeds, like cottonseed hulls, are low in mineral elements, it is thought that prickly-pear is a valuable supplement to such feeds. Cow 5, a strong, vigorous, high-producing animal, when fed a ration with cottonseed hulls as the sole roughage, became so ill in about 10 weeks that it was necessary to change her ration. She showed an abnormal appetite for common salt (sodium chlorid) and upon moving about groaned as if in pain. The treatment ordinarily administered for cases of indigestion afforded no relief, but upon the addition of prickly-pear to her ration she recovered promptly. Cows producing smaller quantities of milk and possessing less robust constitutions, although fed in the same way, remained unaffected. These facts suggest that this cow suffered from a deficiency of mineral elements in her ration.

EFFECT OF FEEDING PRICKLY-PEAR UPON THE OFFSPRING OF DAIRY COWS

In order to determine the effect of prickly-pear upon the offspring, the herd was divided into four groups as soon as the feeding experiment was finished. The first group was fed sorghum hay alone as a roughage; the second, sorghum hay with a medium amount of prickly-pear; the third, sorghum hay with a large quantity of prickly-pear; and the fourth, prickly-pear alone. Grain was fed in such amounts as were necessary to put the cows in good condition at the time of calving. The grain ration of the cows receiving prickly-pear alone as roughage consisted entirely of cottonseed meal; the cows in the other groups received the same grain mixture that was used in the feeding experiment. Except for a period of two or three weeks, when some of the animals were on digestion trials, these rations were continued until the cows freshened, making a period of about five months for each cow.

Table XVI gives the numbers of the cows in each group, the character of their rations, the length of the gestation period, weight of the dam, the sex and weight of the calf, and some brief notes on the condition of the calf at birth.

TABLE XVI.—*Effect of feeding prickly-pear upon the offspring of dairy cows*

No. of cow.	Roughage ration.	Length of gestation period.	Weight of dam.	Sex of calf.	Birth weight of calf.	Condition of calf.
		Days.	*Pounds.*		*Pounds.*	
1.....	Prickly-pear alone..............	274	912	Male....	59	Fair.
13....	.....do.....................	279	696	Female .	48	Weak, died of white scours.
3.....	Heavy prickly-pear.............	272	870	{Male....	48	{Twin calves, both small
				{Female .	39	{ but vigorous.
5.....	.....do.....................	276	907	Female .	60	Fair.
4.....	Medium prickly-pear...........	281	920	...do.....	57	Big-boned, weak.
6.....	.....do.....................	273	890	...do.....	57	Strong.
10....	.....do.....................	279	700	...do.....	59	Do.
11....	.....do.....................	277	762	...do.....	58	Do.
8.....	No prickly-pear...............	273	809	Male....	56	Fair.
9.....	.....do.....................	272	800	Female .	58	Small-boned, vigorous.
12....	.....do.....................	274	806	Male....	45	Small, weak, grew well.

All the cows, except No. 13, were in good condition at the time of calving, and their general health returned to normal within a short time. A small part of the afterbirth was retained for 48 hours by cow 3.

It may be noted that all the calves were rather light in weight as compared with their dams at the time of parturition, but only a few were lacking in vigor. The calves were taken from their dams within 24 hours after birth, and all received the same feed and care. With the exception of the calf from cow 13, they grew normally and no trouble was experienced from scours or from any other calf diseases. Cow 13 calved about three weeks later than any of the others. She was in poor condition at the time, as she had been getting a ration with prickly-pear as the sole roughage. Her calf developed white scours and died within a short time.

The data obtained are too meager to admit of positive conclusions, but it appears that prickly-pear has no great influence on the size and vigor of the offspring, at least when supplemented with some dry roughage.

EFFECT OF COMMON SALT ON THE LAXATIVE PROPERTY OF PRICKLY-PEAR

Some feeders of prickly-pear in southern Texas make a practice of adding common salt to the ration, with the object of lessening its laxative property. The fact that prickly-pear contains a relatively small quantity of sodium made it appear possible that there is some basis for this belief. In order to test this matter, one cow was fed a daily ration of 150 pounds of prickly-pear, 4 pounds of sorghum hay, and 4 pounds of a grain mixture of corn meal, wheat bran, and cottonseed meal; another cow was fed a ration of 150 pounds of prickly-pear and 4 pounds of cottonseed meal.

Sodium chlorid was scattered over the chopped prickly-pear, using ½ ounce to each cow on the first day and increasing the quantity at the rate of ½ ounce a day until the cows refused to eat the feed. One of the cows refused to eat prickly-pear when 4½ ounces of common salt a day were added to it; the other refused her feed when 6½ ounces had been scattered over the ration; but both animals readily ate unsalted prickly-pear whenever it was offered. Both cows were fed for four days on prickly-pear salted to the maximum amount for each animal. No apparent change in the character of the feces was noticeable as the result of feeding the salted ration. Until the maximum quantity of common salt was reached, both cows seemed to have a better appetite for prickly-pear, and they drank a small quantity of water each morning, which they did not always do when common salt was not fed. This test was repeated, using the same cows and feeding them in the same manner as before, with similar results. The two tests indicate that the addition of sodium chlorid to a ration of prickly-pear will have no appreciable effect on the laxative properties of the plant.

EFFECT OF FEEDING PRICKLY-PEAR ON THE QUANTITY OF WATER DRUNK
BY DAIRY COWS

Prickly-pear grows readily in semiarid regions, where the water supply
for cattle is often a serious problem. It has such a high water content
that it was thought to be of interest to ascertain the quantity of
water drunk by animals fed with prickly-pear. In November, 1911, a
short test was conducted for this purpose; the amount of water drunk
by the different animals was recorded for a period of three days, the cows
being watered twice daily. Later, in May and June, 1912, while con-
ducting some digestion trials, the quantities of water drunk by the
animals used in these trials were also weighed. The results are given in
Table XVII.

TABLE XVII.—*Effect of feeding prickly-pear upon the amount of water drunk by dairy
cows*

Date.	Length of period.	Number of cows.	Feed.	Average daily amount of water drunk by each cow.
	Days.			*Pounds.*
November, 1911	3	3	Heavy prickly-pear	5
Do	3	3	Medium prickly-pear	30
Do	3	3	Sorghum hay	69°
Do	3	1	Prickly-pear alone	0
May, 1912	10	2	Sorghum hay	95
May and June, 1912	10	4	Medium prickly-pear	44. 3
June, 1912	10	2	Heavy prickly-pear	0
May and June, 1912	10	2	Prickly-pear alone	0. 3

The warmer weather at the time the digestion trials were made caused
an increased consumption of water by the hay-fed cows, but not by
those heavily fed with prickly-pear. The results of these two trials
indicate that prickly-pear may be of special value in case of shortage
of water. It is possible that animals fed a heavy or an entire roughage
ration of prickly-pear can subsist for a considerable time without
water. During the 3-day trial the cow getting a roughage ration
entirely of prickly-pear drank no water, and the heavy-ration prickly-
pear group drank but a small quantity. During the longer period the
cows on entire prickly-pear and heavy prickly-pear rations drank very
little water, although it was freely offered to them.

EFFECT OF NORTHERS ON COWS FED WITH PRICKLY-PEAR

Southern Texas, where this experiment was conducted, is subject
during the winter months to sudden and decided drops in temperature,
accompanied by strong north winds, locally called "northers." During
the early part of the winter it was noticed that the cows receiving the
heavier prickly-pear ration were apparently more sensitive to these

sudden changes of temperature. The effect of feeding the different rations during severe weather will be readily noted in Table XVIII and also graphically in figure 1. It will be seen that the cows fed heavy rations of prickly-pear showed an average decrease of 7.50 per cent in their milk production on the days of the northers, the cows fed medium rations a decrease of 4.03 per cent, while the milk flow of cows that received no prickly-pear in their ration decreased but 1.92 per cent. These data indicate that the cows feeding on prickly-pear were more sensitive to cold weather than those receiving hay and that the larger quantity of the plant caused the greater sensitiveness. That the greater decrease in production of the prickly-pear-fed cows is due entirely to the change in temperature is more apparent when it is noted that all the cows returned to practically the same percentage of their normal production on the second day following the northers.
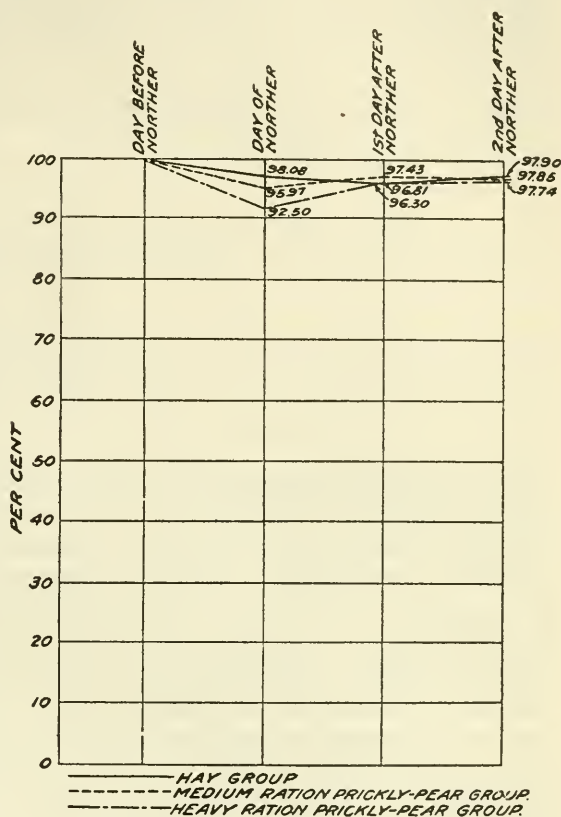
Fig. 1.—Effect of northers upon yield of milk by cows fed with different rations.

TABLE XVIII.—*Effect of 13 northers upon the milk yield of cows fed with prickly-pear and hay*

Group.	Average total milk yield per group.				Percentage of decrease from day before norther.		
	Day before norther.	Day of norther.	First day after norther.	Second day after norther.	Day of norther.	First day after norther.	Second day after norther.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Per cent.*	*Per cent.*	*Per cent.*
Heavy prickly-pear..............	207.9	192.3	200.2	203.2	7.50	3.70	2.26
Medium prickly-pear...........	260.3	249.8	253.6	254.7	4.03	2.57	2.15
Sorghum hay..................	229.1	224.7	221.8	224.3	1.92	3.19	2.10

USE OF PRICKLY-PEAR FOR MAINTENANCE OF DRY COWS

At the close of the first year's work a number of maintenance trials with dry cows were conducted, using the several roughage rations fed during the first year's work. In all these trials cottonseed meal was the only grain fed. A summary of the results is given in Table XIX.

TABLE XIX.—*Results of tests showing the value of prickly-pear as a maintenance ration for dry dairy cows*

No. of cow.	Length of period.	Initial weight.	Final weight.	Average daily amount of feed consumed by each cow.		
				Grain.	Sorghum hay.	Prickly-pear.
	Days.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
3	50	867.4	867.0	1	3.5	105
6	70	796.8	795.6	1	4.0	60
4	50	884.6	885.8	1	6.2	60
13	60	754.8	754.4	2	0	105
1	50	891.6	891.2	2	0	113
13	70	702.0	671.8	0	0	126

In conducting the trials in which both prickly-pear and sorghum hay were fed, the cacti as well as the grain were supplied in fixed quantities, and the sorghum hay was fed in addition in such amounts as to control the body weights. In those trials where prickly-pear was the sole roughage, 120 pounds of prickly-pear a day were fed at the beginning; but later it was found necessary to reduce this quantity to control the body weights. The results of these trials show that mature Jersey cows can be maintained on a ration of 3.5 to 6 pounds of sorghum hay and 60 to 105 pounds of prickly-pear, with 1 pound of cottonseed meal a day. If prickly-pear is used as the sole roughage, it will require about 110 pounds of that plant with an increase of cottonseed meal to 2 a day.

In order to ascertain the possibility of using prickly-pear without supplementary feed as a maintenance ration a second trial was conducted with cow 13. Prickly-pear was fed to this cow in as large quantities as she would consume, the feeding being continued in this manner for 70 days. This cow was in poor condition at the beginning of the 70-day period, and during the period she lost 30.2 pounds. It appears from this trial that prickly-pear alone is not a satisfactory maintenance ration, but that it will keep an animal alive for a considerable time where there is a shortage of or total absence of other feed.

METHOD AND COST OF HARVESTING PRICKLY-PEAR

The cost of harvesting prickly-pear can be only approximately determined, as so much depends upon local conditions. Before the spiny varieties of prickly-pear can be fed they must be treated in such manner as either to remove or soften the spines. The most common method of

removing them is by means of singeing with a strong gasoline torch, as shown in Plate LXI, figure 1, or by burning the spines off over a brush fire, if but a small quantity of prickly-pear is to be fed. Chopping machines have been used with some success to render the spines practically harmless, but the practice of singeing with the gasoline torch is economical in both labor and the greater utility of the prepared feed (4, p. 13). By using the gasoline torch in an average of three trials at Brownsville, Tex., by the Bureau of Animal Industry, it was found that 50 minutes' time and 1⅔ gallons of gasoline were required to singe 1 ton of the spiny prickly-pear. These trials were conducted with a 2-year growth of the plant that yielded at the rate of about 80 tons an acre per annum. With the class of labor obtainable and the price of gasoline at that time, it was estimated that prickly-pear could be singed at a cost of approximately 50 cents a ton.

There are two methods of feeding prickly-pear from which the spines have been removed: The cattle may graze the standing plant down to the heavier stems or it may be cut down and hauled to the feed lots. The first method requires less labor, but is more wasteful and is not advisable, especially with a cultivated plantation, unless the supply of prickly-pear is plentiful. If the cacti are removed to feed lots, the cost of feeding will depend upon the proximity of the lots and the accommodations for feeding. One man with a team can haul and feed from 3 to 6 tons a day (Pl. LXI, fig. 2, and LXII, fig. 1).

A test on a small scale showed that prickly-pear was not suitable for making silage. At the end of 30 days only the small spines had been softened; furthermore, the cows would not eat it.

COMPARATIVE VALUE OF SPINY AND SPINELESS PRICKLY-PEAR

The spineless varieties of prickly-pear are relatively free from thorns or spines. They have practically the same chemical composition as the spiny varieties and are probably of equal value for feeding purposes. However, they are less hardy than the spiny varieties and more subject to injury from low temperatures, so that the area in which they can be successfully grown is much more restricted than that of the spiny varieties. But little accurate information is obtainable as to the yield of the spineless varieties. In work conducted at Chico, Cal., by one of the writers (6, p. 9) an annual yield of from 20 to 25 tons an acre was obtained. These yields were obtained with expert cultivation and by maintaining a perfect stand. At Brownsville, Tex., where the work reported in this paper was conducted, there is no authentic record of the yield of the spineless varieties. It is known, however, that because of insects and low temperature the yield is much less than that of the native spiny forms.

The spineless and the spiny varieties are apparently of equal value for milk production. During the latter part of the second year's work the spineless prickly-pear was substituted for the spiny in the ration of all

the cows fed with this plant. There is apparently some difference in taste between the raw spineless and the singed spiny prickly-pear, as some of the cows ate the former with somewhat less relish for the first few days after the change was made. This was likewise true when the spiny prickly-pear was again fed, but in both cases the two varieties were eaten with equal relish after the first few days of feeding. No change in production or body condition was caused by the change in the kind of prickly-pear fed, and the spineless had the same laxative effect as the spiny variety.

The cost of harvesting and feeding the spineless kind would differ from that of the spiny varieties only in the cost of singeing. The spineless prickly-pear, unlike the spiny form, could not be harvested by grazing, for the amount to be fed daily could not be controlled, as is the case with the spiny forms. The waste of feed and destruction of plants by stock in the field would be so great as to make that method of harvesting less economical than cutting and hauling the cacti to a feed lot.

SUMMARY

The average analysis of prickly-pear fed in these experiments was as follows: Water, 91.30 per cent; crude protein (N × 6.25), 0.58 per cent; albuminoid protein, 0.29 per cent; ether extract, 0.12 per cent; nitrogen-free extract, 4.67 per cent; crude fiber, 1.16 per cent; ash, 1.76 per cent.

Prickly-pear was found to be a very palatable feed for dairy cows, even when it formed the major part of the roughage ration, and 100 to 150 pounds were consumed per cow per day.

The prickly-pear ration caused an increase in the quantity of milk produced, a decrease in the percentage of fat in the milk, and a decrease in the total production of fat. The reduction in the percentage of fat became more pronounced as the quantity of prickly-pears in the ration increased.

Assuming the feeds to have these percentages of dry matter—prickly-pear, 10; sorghum hay, 80; sorghum silage, 25; and cottonseed hulls, 90—and considering the nutritive values to vary in direct proportion to the content of dry matter, 1 pound of sorghum hay was equal to 15.9 pounds of prickly-pear when that plant was fed in large quantities and to 10.1 pounds of prickly-pear when it was fed in moderate amounts. One pound of sorghum silage was equal to 2.6 pounds of prickly-pear, and 1 pound of cottonseed hulls was equal to 5.8 pounds of prickly-pear.

When prickly-pear in moderate amounts was substituted for a part of the dry roughage, it appeared to have little effect on the digestion of the other ingredients of the ration; when substituted in large amounts it depressed the coefficient of digestion, although not to any great extent.

As the result of maintenance trials conducted during these experiments, it is believed that mature Jersey cows can be maintained on a daily

ration of 3.5 to 6 pounds of sorghum hay, 60 to 100 pounds of prickly-pear, and 1 pound of cottonseed meal a day; or, with prickly-pear as the sole roughage, about 110 pounds of that plant and 2 pounds of cotton-seed meal. Prickly-pear alone did not make a satisfactory maintenance ration, but sustained life for a long time. One cow that was fed prickly-pear alone for a period of 70 days lost 30.2 pounds live weight.

The average coefficients of digestion in two trials with prickly-pear as the sole ration were as follows: Dry matter, 61.58; ash, 38.37; crude protein, 71.56; crude fiber, 42.98; nitrogen-free extract, 71.55; ether extract, 65.88; organic matter, 67.21.

Palatability was apparently an important factor in feeding prickly-pears as the sole roughage. One cow that ate prickly-pear with relish did as well on the ration when that plant was the sole roughage as when some dry roughage was included. Another that ate prickly-pear reluctantly lost in weight. In one case feeding prickly-pear alone caused the formation of an obstruction in the intestine and the death of the animal.

The feeding of prickly-pear produced a highly colored butter, but had no appreciable effect upon the flavor or keeping quality of the milk.

Prickly-pear had a decidedly laxative effect on the cows, although there seemed to be no permanent ill effects even after long-continued feeding. The addition of common salt (sodium chlorid) to a ration of prickly-pear even when added in large amounts, 4 to 6 ounces a day to each cow, had no appreciable effect upon the laxative property of the plant.

During an experimental period of 10 days cows receiving a heavy ration of prickly-pear drank no water, those receiving a medium ration drank an average of 44.3 pounds of water per day, while those on a roughage ration of sorghum hay drank a daily average of 95 pounds.

As measured by milk production cows fed prickly-pear were more sensitive to northers than those which received a dry roughage. The greater the quantity of the plant consumed the more sensitive the animal became.

The prickly-pear ration appeared to have no great influence upon the size and vigor of the offspring or upon the condition of the cow after parturition.

The cost of harvesting prickly-pear depends largely upon local conditions. During these experiments it was found that the spines could be singed at a cost of about 50 cents per ton.

There was no great difference between the spineless and the spiny varieties of prickly-pear in composition, palatability, or feeding value. While the cost of harvesting the spineless was less than that of the spiny varieties, the latter yielded a greater tonnage to the acre at Brownsville, Tex., and were not so subject to injury from insects. The spiny varieties are hardier and can be grown in a much greater area than the spineless.

These experiments were conducted for periods long enough to show conclusively that prickly-pear is a good and palatable feed for dairy cows. It is best to feed the plant in medium quantities, 60 to 75 pounds a day to each cow. When fed in large amounts, 120 to 150 pounds a day, it causes an excessive scouring and a very insanitary condition of the stable. On account of the high content of mineral matter, it is thought that prickly-pear may be of special value as a supplementary feed for use with other roughages of a low mineral-matter content, such as cottonseed hulls.

LITERATURE CITED

(1) ARMSBY, H. P.
    1909. The computation of rations for farm animals by the use of energy values. U. S. Dept. Agr. Farmers' Bul. 346, 32 p.

(2) ECKLES, C. H.
    1913. Nutrients required for milk production. Mo. Agr. Exp. Sta. Research Bul. 7, 140 p.

(3) FARRINGTON, E. H., and WOLL, F. W.
    1911. Testing milk and its products. ed. 20, 297 p., 61 fig., 25 tab. Madison, Wis.

(4) GRIFFITHS, David.
    1905. The prickly pear and other cacti as food for stock. U. S. Dept. Agr. Bur. Plant Indus. Bul. 74, 46 p., 5 pl.

(5) ————
    1906. Feeding prickly pear to stock in Texas. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 91, 23 p., 3 pl.

(6) ————
    1912. The thornless prickly pears. U. S. Dept. Agr. Farmers' Bul. 483, 20 p., 4 fig.

(7) HARE, R. F.
    1908. Experiments on the digestibility of prickly pear by cattle. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 106, 38 p., 1 fig., 1 pl.

(8) HENRY, W. A.
    1910. Feeds and feeding . . . ed. 10, 613 p. Madison, Wis.

(9) MEHTA, P. R.
    1904. Prickly-pear and aloe as fodder for cattle during scarcity. Dept. Land Records and Agr. Bombay, Bul. 22, 1903, 5 p.

(10) SOTGIA, Giuseppe.
    1900. Studio chimico-zootecnico circa il razionale impiego delle palette di fico d'India nell' alimentazione delle vacche da latte in Sardegna. *In* Staz. Sper. Agr. Ital., v. 33, fasc. 2, p. 113–167.

TABLE XX.—*Milk production (first year): Prickly-pear versus sorghum hay*

FIRST PERIOD (80 DAYS)

No. of cow.	Group.	Total milk.	Fat in milk.		Average specific gravity.	Total solids.
			Average per cent.	Total amount.		
		Pounds.	*Per cent.*	*Pounds.*		*Pounds.*
1	Heavy prickly-pear	806. 7	3. 88	31. 34	1. 0327	103. 62
2	do	1,426. 4	3. 83	54. 57	1. 0322	180. 52
3	do	1,482. 9	3. 98	58. 96	1. 0326	191. 83
Total		3,716. 0	3. 90	144. 87	....	475. 97
4	Medium prickly-pear	1,332. 0	4. 92	65. 49	1. 0327	187. 77
5	do	2,093. 6	4. 15	86. 97	1. 0324	274. 14
6	do	1,251. 0	4. 32	54. 07	1. 0332	168. 91
Total		4,676. 6	4. 42	206. 53	....	630. 82
8	Medium prickly-pear	2,055. 1	3. 91	80. 27	1. 0312	256. 80
9	do	1,488. 0	3. 68	54. 83	1. 0328	188. 01
Total		3,543. 1	3. 81	135. 10	....	444. 81
10	Sorghum hay	1,394. 4	4. 24	59. 06	1. 0329	185. 95
11	do	1,734. 4	4. 52	78. 34	1. 0325	235. 30
12	do	1,482. 5	5. 88	87. 15	1. 0347	233. 62
Total		4,611. 3	4. 87	224. 55	....	654. 87
13	Prickly-pear alone	575. 6	5. 06	29. 11	1. 0322	81. 37

SECOND PERIOD (80 DAYS)

No. of cow.	Group.	Total milk.	Average per cent.	Total amount.	Average specific gravity.	Total solids.
1	Medium prickly-pear	637. 6	3. 87	24. 67	1. 0314	79. 69
2	do	1,288. 4	4. 09	52. 65	1. 0317	165. 38
3	do	1,280. 8	4. 34	55. 59	1. 0325	170. 99
Total		3,206. 8	4. 14	132. 91	....	416. 06
4	Heavy prickly-pear	1,132. 1	4. 35	49. 25	1. 0321	150. 12
5	do	2,248. 2	3. 76	84. 54	1. 0321	282. 16
6	do	1,062. 0	3. 86	40. 97	1. 0327	136. 13
Total		4,442. 3	3. 93	174. 76	....	568. 41
8	Sorghum hay	1,757. 9	4. 11	72. 25	1. 0303	220. 09
9	do	1,472. 9	3. 97	58. 44	1. 0324	189. 53
Total		3,230. 8	4. 04	130. 69	....	409. 62
10	Medium prickly-pear	1,230. 9	3. 78	46. 49	1. 0325	155. 92
11	do	1,622. 9	3. 77	61. 24	1. 0319	203. 08
12	do	1,532. 0	5. 37	82. 25	1. 0341	229. 78
Total		4,385. 8	4. 33	189. 98	....	588. 78
13	Prickly-pear alone	392. 0	5. 47	21. 44	1. 0312	56. 37

TABLE XXI.—*Milk production (second year): Prickly-pear versus sorghum silage*

FIRST PERIOD (80 DAYS)

| No. of cow. | Group. | Total milk. | Fat in milk. | | Average specific gravity. | Total solids. |
			Average percent-age.	Total amount.		
		Pounds.	*Per cent.*	*Pounds.*		*Pounds.*
3..............	Sorghum silage...................	1,660. 0	4. 49	74. 84	1. 0321	223. 24
14..............	....do...........................	1,183. 5	5. 00	59. 26	1. 0332	169. 56
17..............	....do...........................	1,889. 3	5. 13	97. 05	1. 0335	275. 02
19..............	....do...........................	1,795. 6	5. 60	100. 57	1. 0339	273. 51
20..............	....do...........................	1,110. 1	5. 51	61. 14	1. 0321	162. 60
Total.....		7,638. 5	5. 14	392. 86	....	1,103. 93
6..............	Prickly-pear......................	1,640. 4	4. 39	72. 11	1. 0331	222. 21
9..............	....do...........................	2,364. 5	3. 96	93. 70	1. 0311	295. 86
15..............	....do...........................	1,457. 1	4. 12	60. 04	1. 0316	186. 35
16..............	....do...........................	1,830. 1	4. 03	73. 78	1. 0326	237. 94
18..............	....do...........................	1,512. 0	5. 16	78. 05	1. 0322	215. 34
Total.....		8,804. 1	4. 29	377. 68	....	1,157. 70

SECOND PERIOD (80 DAYS)

No. of cow.	Group.	Total milk.	Average percentage.	Total amount.	Average specific gravity.	Total solids.
3..............	Prickly-pear......................	1,642. 9	4. 12	67. 71	1. 0338	220. 17
14..............	....do...........................	1,060. 1	4. 74	50. 34	1. 0341	150. 73
17..............	....do...........................	1,763. 8	4. 79	84. 46	1. 0349	255. 72
19..............	....do...........................	1,520. 2	5. 26	79. 96	1. 0351	229. 40
20..............	....do...........................	830. 6	4. 92	40. 94	1. 0340	119. 85
Total.....		6,817. 6	4. 74	323. 41	....	975. 87
6..............	Sorghum silage...................	1,310. 8	5. 02	65. 87	1. 0347	193. 02
9..............	....do...........................	1,990. 1	4. 08	81. 36	1. 0335	264. 25
15..............	....do...........................	1,083. 1	4. 42	47. 91	1. 0343	150. 59
16..............	....do...........................	1,976. 7	4. 46	88. 28	1. 0340	274. 29
18..............	....do...........................	1,288. 3	6. 01	77. 50	1. 0339	202. 59
Total.....		7,649. 0	4. 72	360. 92	....	1,084. 74

TABLE XXII.—*Milk production (second year): Prickly-pear versus cottonseed hulls*

FIRST PERIOD (80 DAYS)

| No. of cow. | Group. | Total milk. | Fat in milk. | | Average specific gravity. | Total solids. |
			Average percent-age.	Total amount.		
		Pounds.	*Per cent.*	*Pounds.*		*Pounds.*
4..............	Cottonseed hulls.................	1,771. 4	5. 18	91. 86	1. 0323	253. 77
5 [a]..............	....do...........................	2,498. 1	5. 03	125. 83	1. 0328	356. 15
10..............	....do...........................	1,805. 7	4. 44	80. 31	1. 0334	247. 52
Total.....		6,075. 2	4. 90	298. 00	....	857. 44
8..............	Prickly-pear......................	2,340. 3	3. 95	92. 56	1. 0311	293. 12
11..............	....do...........................	2,100. 2	4. 17	87. 69	1. 0330	278. 81
12..............	....do...........................	1,913. 9	5. 72	109. 55	1. 0358	303. 33
Total.....		6,354. 4	4. 56	289. 80	....	875. 26
1..............	Prickly-pear alone................	1,089. 2	4. 11	44. 75	1. 0331	143. 87
13 [b]..............	....do...........................	24. 1	4. 56	1. 10	1. 0282	3. 02
Total.....		....	....	....	....	....

[a] Period, 70 days.   [b] Period, 10 days.

TABLE XXII.—*Milk production (second year): Prickly-pear versus cottonseed hulls*—Con.

SECOND PERIOD (80 DAYS)

| No. of cow. | Group. | Total milk. | Fat in milk. | | Average specific gravity. | Total solids. |
			Average percentage.	Total amount.		
		Pounds.	*Per cent.*	*Pounds.*		*Pounds.*
4..............	Prickly-pear......................	1,270.6	4.75	60.37	1.0331	177.52
5 [a]..............	.....do........................	2,071.0	4.42	91.59	1.0329	280.67
10..............	.....do........................	1,208.6	4.11	49.70	1.0333	160.29
Total.....	....	4,550.2	4.43	201.66	....	618.48
8..............	Cottonseed hulls.................	1,744.7	4.23	73.91	1.0307	222.73
11..............	.....do........................	1,683.4	4.46	75.18	1.0330	229.38
12..............	.....do........................	1,761.7	6.19	109.11	1.0350	285.28
Total.....	....	5,189.8	4.97	258.20	....	737.39
1..............	Prickly-pear alone................	981.8	3.83	37.66	1.0328	125.83

[a] Period, 70 days.

TABLE XXIII.—*Feeds and body weights (first year): Prickly-pear versus sorghum hay*

FIRST PERIOD (80 DAYS)

| No. of cow. | Group. | Feed consumed. | | | Body weight. | | |
		Grain.	Sorghum hay.	Prickly-pear.	Initial weight.	Final weight.	Gain.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
1..............	Heavy prickly-pear	314.0	296.5	11,629	823.6	833.8	10.2
2..............	.....do.................	513.0	433.5	11,608	687.8	720.2	32.4
3..............	.....do.................	553.0	447.5	11,214	751.6	773.8	22.2
Total.....	....	1,380.0	1,777.5	34,451	2,263.0	2,327.8	64.8
4..............	Medium prickly-pear....	622.0	717.5	5,915	766.4	780.0	13.6
5..............	.....do.................	792.0	1,016.5	5,940	725.2	772.0	46.8
6..............	.....do.................	509.0	667.0	5,940	723.6	757.4	33.8
Total.....	....	1,923.0	2,401.0	17,795	2,215.2	2,309.4	94.2
8..............	Medium prickly-pear....	752.4	676.5	5,940	712.0	753.0	41.0
9..............	.....do.................	535.6	645.0	5,940	603.5	636.8	33.3
Total.....	....	1,288.0	1,321.5	11,880	1,315.5	1,389.8	74.3
10..............	Sorghum hay...........	566.2	1,250.0	....	567.5	608.0	40.5
11..............	.....do.................	729.4	1,358.0	....	676.6	701.6	25.0
12..............	.....do.................	793.0	1,557.5	....	717.0	743.0	26.0
Total.....	....	2,088.6	4,165.5	....	1,961.1	2,052.6	91.5
13..............	Prickly-pear alone.......	[a] 320.0	....	10,069	736.6	728.0	−8.6

[a] Cottonseed meal.

TABLE XXIII.—*Feeds and body weights (first year): Prickly-pear versus sorghum hay—*
Continued

SECOND PERIOD (80 DAYS)

No. of cow.	Group.	Feed consumed.			Body weight.		
		Grain.	Sorghum hay.	Prickly-pear. [a]	Initial weight.	Final weight.	Gain.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
1	Medium prickly-pear....	268.2	612.0	4,800	782.0	774.0	− 8.0
2	do	570.4	771.0	4,800	690.8	680.4	−10.4
3	do	614.0	730.5	4,800	743.8	758.8	15.0
Total		1,452.6	2,113.5	14,400	2,216.6	2,213.2	− 3.4
4	Heavy prickly-pear	508.4	285.5	8,567	804.4	812.2	7.8
5	do	863.0	427.5	9,983	813.8	832.6	18.8
6	do	419.0	282.5	8,698	771.0	776.8	5.8
Total		1,790.0	995.5	27,248	2,389.2	2,421.6	32.4
8	Sorghum hay	771.4	1,192.0	....	751.2	728.0	−23.2
9	do	607.7	1,204.5	....	646.0	653.2	7.2
Total		1,379.1	2,396.5	....	1,397.2	1,381.2	−16.0
10	Medium prickly-pear....	464.4	652.5	4,800	610.4	624.2	13.8
11	do	615.2	635.0	4,800	712.0	713.8	1.8
12	do	809.3	762.0	4,800	756.8	763.6	6.8
Total		1,888.9	2,049.5	14,400	2,079.2	2,101.6	22.4
13	Prickly-pear alone	320.0	....	9,031	729.4	716.2	−13.2

TABLE XXIV.—*Feeds and body weights (second year): Prickly-pear versus sorghum silage*

FIRST PERIOD (80 DAYS)

No. of cow.	Group.	Feed consumed.				Body weight.		
		Grain.	Cotton-seed hulls.	Prickly-pear.	Sorghum silage.	Initial weight.	Final weight.	Gain.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
3	Sorghum silage..	731	800	....	2,274.0	731.8	777.8	46.0
14	do	578	800	....	1,963.0	799.0	851.8	52.8
17	do	953	800	....	2,182.5	764.4	789.4	25.0
19	do	1,017	674	....	2,065.0	767.2	791.6	24.4
20	do	615	800	....	2,365.0	885.8	905.4	19.6
Total		3,894	3,874	....	10,849.5	3,948.2	4,116.0	167.8
6	Prickly-pear, ...	733	800	6,000	....	788.8	819.6	30.8
9	do	928	800	6,000	....	719.6	737.4	17.8
15	do	636	686	5,975	....	858.2	868.6	10.4
16	do	732	800	6,000	....	771.8	807.4	35.6
18	do	782	800	6,000	....	797.8	837.8	40.0
Total		3,811	3,886	29,975	....	3,936.2	4,070.8	134.6

SECOND PERIOD (80 DAYS)

No. of cow.	Group.	Grain.	Cotton-seed hulls.	Prickly-pear.	Sorghum silage.	Initial weight.	Final weight.	Gain.
3	Prickly-pear....	690.4	740	5,881	....	809.4	783.4	−26.0
14	do	508.8	640	5,732	....	887.8	897.8	10.0
17	do	865.8	720	5,852	....	815.4	824.8	9.4
19	do	814.7	640	5,775	....	826.8	824.2	− 2.6
20	do	422.2	800	6,000	....	919.6	932.0	12.4
Total		3,301.9	3,540	29,240	....	4,259.0	4,262.2	3.2
6	Sorghum silage..	673.6	852	....	1,632	797.8	805.8	8.0
9	do	840.0	732	....	1,664	719.0	720.0	1.0
15	do	493.5	730	....	1,679	861.4	841.6	−19.8
16	do	886.7	852	....	1,876	810.2	804.2	− 6.0
18	do	791.3	832	....	1,648	839.8	823.6	−16.2
Total		3,685.1	3,998	....	8,499	4,028.2	3,995.2	−33.0

TABLE XXV.—*Feeds and body weights (second year): Prickly-pear alone versus cotton-seed hulls*

FIRST PERIOD (80 DAYS)

No. of cow.	Group.	Feed consumed.			Body weight.		
		Grain.	Cotton-seed hulls.	Prickly-pear.	Initial weight.	Final weight.	Gain.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
4............	Cottonseed hulls	1,012	1,540	....	854.4	853.2	−1.2
5[a]............	.....do	1,318.7	1,492	....	863.8	870.2	6.4
10............	.....do	890	1,545	....	693.8	713.2	19.4
Total..	....	3,220.7	4,577	....	2,412.0	2,436.6	24.6
8............	Prickly-pear alone........	1,035	720	6,000	781.6	803.8	22.2
11............	.....do	977	640	5,080	803.6	803.0	−.6
12............	.....do	1,169	720	6,000	765.8	799.2	33.4
Total..	....	3,181	2,080	17,080	2,351.0	2,406.0	55.0
1............	Prickly-pear alone.[b]......	400	....	11,945	849.8	861.2	11.4
13............	.....do	....	....	9,919	695.8	671.8	−24.0

SECOND PERIOD (80 DAYS)

4............	Prickly-pear alone........	640	710	5,960	866.0	878.8	12.8
5[a]............	.....do	919	682	5,250	862.8	885.6	22.8
10............	.....do	527	492	5,467	701.0	705.0	4.0
Total..	....	2,086	1,884	16,677	2,429.8	2,469.4	39.6
8............	Cottonseed hulls	771	1,481	....	779.0	803.6	24.6
11............	.....do	789	1,348	....	776.6	805.2	28.6
12............	.....do	1,123	1,415	....	793.6	811.0	17.4
Total..	....	2,683	4,244	....	2,349.2	2,419.8	70.6
1............	Prickly-pear alone.[b]......	400	[c] 1,350	10,640	844.2	850.8	6.6

[a] 70 days only.   [b] Cottonseed meal.   [c] Spineless cactus.

TABLE XXVI.—*Analyses of feeds used in prickly-pear feeding experiments*

FIRST YEAR (FIRST PERIOD; 80 DAYS)

Feed.	Moisture.	Ash.	Total crude protein (N×6.25).	Albumi-noid protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Corn chop....................	12.00	1.45	9.31	9.12	2.39	70.93	3.92
Wheat bran..................	10.40	5.95	17.72	15.25	8.35	52.95	4.63
Cottonseed meal..............	8.68	6.40	45.72	43.09	6.53	20.54	12.13
Sorghum hay	30.36	6.79	4.94	4.16	23.73	32.46	1.72
Prickly-pear.................	90.96	2.27	.90	.406	1.73	4.02	.12

FIRST YEAR (SECOND PERIOD; 80 DAYS)

Corn chop....................	11.66	1.68	9.53	9.25	3.55	70.07	3.51
Wheat bran..................	8.98	5.86	17.81	15.17	8.15	54.02	5.18
Cottonseed meal..............	8.44	6.28	43.83	41.33	5.21	18.84	17.40
Sorghum hay.................	6.15	7.84	4.47	3.97	27.71	51.73	2.10
Prickly-pear.................	88.00	2.09	.83	.34	1.23	6.08	.19

TABLE XXVI.—*Analyses of feeds used in prickly-pear feeding experiments*—Continued

SECOND YEAR (FIRST PERIOD; 80 DAYS)

Feed.	Moisture.	Ash.	Total crude protein ($N \times 6.25$).	Albuminoid protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Corn chop....................	9.81	1.37	10.76	10.48	1.89	72.19	3.98
Bran.......................	7.16	4.94	19.32	16.35	6.65	58.75	3.13
Cottonseed meal..............	5.66	6.51	45.38	42.74	4.41	30.67	7.37
Cottonseed hulls.............	6.59	3.88	4.07	4.04	45.66	38.65	1.15
Sorghum silage..............	78.12	2.35	1.74	1.22	6.56	10.36	.87
Prickly-pear.................	92.58	1.48	.29	.22	.89	4.67	.087

SECOND YEAR (SECOND PERIOD; 80 DAYS)

Feed.	Moisture.	Ash.	Total crude protein.	Albuminoid protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
Corn chop....................	9.59	1.64	9.66	9.54	2.14	74.66	2.26
Bran.......................	8.57	5.09	19.45	17.55	6.73	57.06	3.10
Cottonseed meal..............	11.31	5.71	46.66	41.24	6.41	20.76	9.15
Cottonseed hulls.............	6.47	2.98	4.14	3.93	46.31	39.35	.75
Sorghum silage..............	78.06	2.57	1.73	1.04	5.60	10.93	1.11
Prickly-pear.................	93.67	1.22	.313	.205	.80	3.91	.092

TABLE XXVII.—*Nutrients consumed: Group totals (first year)*

FIRST PERIOD (80 DAYS)

Feed.	Nutrient.						Total.
	Dry matter.	Crude protein ($N \times 6.25$).	Crude fiber.	Nitrogen-free extract.	Ether extract.	Amids.	
Heavy prickly-pear group (cows 1, 2, 3):	Pounds.	Pounds.	Pounds.	Pounds.	Pounds.	Pounds.	Pounds.
Corn chop...................	404.80	42.83	10.99	326.28	18.03	0.87	803.80
Wheat bran.................	412.16	81.51	38.41	243.57	21.30	11.36	808.31
Cottonseed meal.............	420.07	210.31	30.04	94.48	55.80	12.10	822.80
Sorghum hay.................	820.01	58.17	279.42	388.22	20.25	9.18	1,569.25
Prickly-pear.....	3.114.37	310.06	596.00	1,384.93	41.34	168.81	5,615.51
Total...................	5,171.41	702.88	954.86	2,431.48	156.72	202.32	9,619.67
Medium prickly-pear group (cows 4, 5, 6):							
Corn chop..................	564.08	59.68	15.32	454.66	25.13	1.22	1,120.09
Wheat bran.................	574.34	113.59	53.52	339.41	29.68	15.83	1,126.37
Cottonseed meal.............	585.36	293.07	41.86	131.66	77.75	16.86	1,146.56
Sorghum hay.................	1,672.06	118.61	569.76	779.36	41.30	18.73	3,199.82
Prickly-pear	1,608.67	160.16	307.85	715.36	21.35	87.20	2,900.59
Total...................	5,004.51	745.11	988.31	2,420.45	195.21	139.84	9,493.43
Medium prickly-pear group (cows 8, 9):							
Corn chop..................	377.78	39.97	10.26	304.50	16.83	.82	750.16
Wheat bran.................	384.65	76.07	35.85	227.31	19.88	10.60	754.36
Cottonseed meal.............	392.04	196.28	28.03	88.18	52.07	11.29	767.89
Sorghum hay.................	920.29	65.28	313.59	428.96	22.73	10.31	1,761.16
Prickly-pear	1,073.95	106.92	205.52	477.58	14.26	58.21	1,936.44
Total...................	3,148.71	484.52	593.25	1,526.53	125.77	91.23	5,970.01
Sorghum-hay group (cows 10, 11, 12):							
Corn chop..................	612.66	64.82	16.64	493.81	27.29	1.32	1,216.54
Wheat bran.................	623.80	123.37	58.13	368.64	32.23	17.20	1,223.37
Cottonseed meal.............	635.77	318.30	45.46	143.00	84.45	18.31	1,245.29
Sorghum hay.................	2,900.85	205.78	988.47	1,352.12	71.65	32.49	5,551.36
Total...................	4,773.08	712.27	1,108.70	2,357.57	215.62	69.32	9,236.56

TABLE XXVII.—*Nutrients consumed: Group totals (first year)*—Continued

SECOND PERIOD (80 DAYS)

Feed.	Nutrient.						Total.
	Dry matter.	Crude protein (N×6.25).	Crude fiber.	Nitrogen-free extract.	Ether extract.	Amids.	
Heavy prickly-pear group (cows 4, 5, 6):	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Corn chop	527.12	56.87	21.18	418.11	20.94	1.67	1,045.89
Wheat bran	543.12	106.27	48.63	322.34	30.91	15.75	1,067.02
Cottonseed meal	546.34	261.53	31.09	112.42	103.83	14.92	1,070.13
Sorghum hay	934.28	44.50	275.85	514.97	20.91	4.98	1,795.49
Prickly-pear	3,269.08	226.16	334.69	1,656.68	52.53	132.94	5,672.08
Total	5,819.94	695.33	711.44	3,024.52	229.12	170.26	10,650.61
Medium prickly-pear group (cows 1, 2, 3):							
Corn chop	427.74	46.14	17.19	339.28	17.00	1.36	848.71
Wheat bran	440.72	86.24	39.46	261.56	25.08	12.78	865.84
Cottonseed meal	443.34	212.22	25.23	91.22	84.25	12.11	868.37
Sorghum hay	1,983.52	94.47	585.65	1,093.31	44.38	10.57	3,811.90
Prickly-pear	1,727.64	119.52	176.88	875.52	27.76	70.26	2,997.58
Total	5,022.96	558.59	844.41	2,660.89	198.47	107.08	9,392.40
Medium prickly-pear group (cows 10, 11, 12):							
Corn chop	556.19	60.00	22.35	441.16	22.10	1.76	1,103.56
Wheat bran	573.06	112.13	51.31	340.11	32.61	16.62	1,125.84
Cottonseed meal	576.46	275.95	32.80	118.62	109.55	15.74	1,129.12
Sorghum hay	1,923.46	91.61	567.92	1,060.21	43.04	10.25	3,696.49
Prickly-pear	1,727.64	119.52	176.88	875.52	27.76	70.26	2,997.58
Total	5,356.81	659.21	851.26	2,835.62	235.06	114.63	10,052.59
Sorghum-hay group (cows 8, 9):							
Corn chop	406.10	43.81	16.32	322.11	16.14	1.29	805.77
Wheat bran	418.42	81.87	37.47	268.33	23.81	12.14	822.04
Cottonseed meal	420.90	201.49	23.95	86.61	79.99	11.49	824.43
Sorghum hay	2,249.12	107.12	664.07	1,239.71	50.33	11.98	4,322.33
Total	3,494.54	434.29	741.81	1,896.76	170.27	36.90	6,774.57

TABLE XXVIII.—*Nutrients consumed: Group totals (second year)*

FIRST PERIOD (80 DAYS)

Feed.	Nutrient.						Total.
	Dry matter.	Crude protein (N×6.25).	Crude fiber.	Nitrogen-free extract.	Ether extract.	Amids.	
Sorghum-silage group (cows 3, 14, 17, 19, 20):	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Corn chop	1,170.67	139.66	24.53	937.03	51.66	3.63	2,327.18
Wheat bran	1,205.06	250.77	86.32	762.58	40.63	38.55	2,383.91
Cottonseed meal	1,224.53	589.03	57.24	398.10	95.66	34.27	2,398.83
Cottonseed hulls	3,618.70	157.67	1,768.87	1,497.30	44.55	1.16	7,088.25
Sorghum hay	2,373.87	188.78	711.73	1,124.01	94.39	56.42	4,549.20
Total	9,592.83	1,325.91	2,648.69	4,719.02	326.89	134.03	18,747.37
Prickly-pear group (cows 6, 9, 15, 16, 18):							
Corn chop	1,145.68	136.68	24.01	917.03	50.56	3.56	2,277.52
Wheat bran	1,179.35	245.42	84.47	746.30	39.76	37.73	2,333.03
Cottonseed meal	1,198.40	576.46	56.02	389.60	93.62	33.54	2,347.64
Cottonseed hulls	3,629.91	158.16	1,774.35	1,501.94	42.49	1.16	7,108.01
Prickly-pear	2,224.15	86.93	266.78	1,399.83	26.98	20.98	4,025.65
Total	9,377.49	1,203.65	2,205.63	4,954.70	253.41	96.97	18,091.85

TABLE XXVIII.—*Nutrients consumed: Group totals (second year)*—Continued

FIRST PERIOD (80 DAYS)—continued

Feed.	Nutrient.						Total.
	Dry matter.	Crude protein (N×6.25).	Crude fiber.	Nitrogen-free extract.	Ether extract.	Amids.	
Prickly-pear group (cows 8, 11, 12):	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Corn chop	956.28	114.09	20.04	765.43	42.20	2.97	1,901.01
Wheat bran	984.38	204.85	70.51	622.93	33.19	31.49	1,947.35
Cottonseed meal	1,000.29	481.16	46.76	325.19	78.14	27.99	1,959.53
Cottonseed hulls	1,942.93	84.66	949.73	803.92	23.92	.62	3,805.78
Prickly-pear	1,267.34	49.53	152.01	797.64	15.37	11.96	2,293.85
Total	6,151.22	934.29	1,239.05	3,315.11	192.82	75.03	11,907.52
Cottonseed-hulls group (cows 4, 5, 10):							
Corn chop	968.28	115.52	20.29	775.03	42.73	3.01	1,924.86
Wheat bran	996.73	207.42	71.39	630.74	33.60	31.89	1,971.77
Cottonseed meal	1,012.83	487.20	47.35	329.27	79.12	28.34	1,984.11
Cottonseed-hulls	4,275.38	186.28	2,089.86	1,769.01	52.64	1.37	8,374.54
Total	7,253.22	996.42	2,228.89	3,504.05	208.09	64.61	14,255.28

SECOND PERIOD (80 DAYS)

Feed.	Dry matter.	Crude protein (N×6.25).	Crude fiber.	Nitrogen-free extract.	Ether extract.	Amids.	Total.
Sorghum-silage group (cows 6, 9, 15, 16, 18):							
Corn chop	1,110.60	118.66	26.29	917.12	27.76	1.47	2,201.90
Wheat bran	1,123.13	238.92	82.67	700.92	38.08	23.34	2,207.06
Cottonseed meal	1,089.47	573.17	78.74	255.02	112.40	66.58	2,175.38
Cottonseed hulls	3,739.33	165.52	1,851.47	1,573.21	29.98	8.40	7,367.91
Sorghum silage	1,864.68	147.03	475.94	928.94	94.34	58.64	3,569.57
Total	8,927.21	1,243.30	2,515.11	4,375.21	302.56	158.43	17,521.82
Prickly-pear group (cows 3, 14, 17, 19, 20):							
Corn chop	995.05	106.32	23.55	821.71	24.87	1.32	1,972.82
Wheat bran	1,006.28	214.07	74.07	628.00	34.12	20.91	1,977.45
Cottonseed meal	976.12	513.50	70.55	228.48	100.70	59.65	1,949.00
Cottonseed hulls	3,310.96	146.56	1,639.37	1,392.99	26.55	7.43	6,523.86
Prickly-pear	1,850.89	90.64	233.92	1,143.28	26.32	32.16	3,377.21
Total	8,139.30	1,071.09	2,041.46	4,214.46	212.56	121.47	15,800.34
Prickly-pear group (cows 4, 5, 10):							
Corn chop	628.62	67.17	14.88	519.11	15.71	.83	1,246.32
Wheat bran	635.71	135.24	46.79	396.74	21.55	13.21	1,249.24
Cottonseed meal	616.66	324.43	44.57	144.34	63.62	37.69	1,231.31
Cottonseed hulls	1,762.11	78.00	872.48	741.35	14.13	3.96	3,472.03
Prickly-pear	1,055.65	51.70	133.42	652.07	15.01	18.34	1,926.19
Total	4,698.75	656.54	1,112.14	2,453.61	130.02	74.03	9,125.09
Cottonseed-hulls group (cows 8, 11, 12):							
Corn chop	808.54	86.39	19.14	667.68	20.21	1.07	1,603.03
Wheat bran	817.66	173.94	60.19	510.29	27.72	16.99	1,606.79
Cottonseed meal	793.15	417.28	57.32	185.66	81.83	48.47	1,583.71
Cottonseed hulls	3,969.41	175.70	1,965.40	1,670.01	31.83	8.91	7,821.26
Total	6,388.76	853.31	2,102.05	3,033.64	161.59	75.44	12,614.79

TABLE **XXIX.**—*Nutrients digested: Group totals (first year)*

FIRST PERIOD (80 DAYS)

Feed.	Nutrient.						
	Dry matter.	Crude protein (N×6.25).	Crude fiber.	Nitrogen-free extract.	Ether extract.	Amids.	Total.
Heavy prickly-pear group (cows 1, 2, 3):	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Corn chop	346.27	38.41	7.58	263.99	13.96	0.87	671.08
Wheat bran	255.71	74.06	18.74	150.45	12.08	11.36	522.40
Cottonseed meal	304.04	205.98	12.51	64.11	47.21	12.10	645.95
Sorghum hay	447.07	29.51	162.93	202.84	11.84	9.18	863.37
Prickly-pear	1,802.76	261.82	304.90	862.10	24.51	168.81	3,424.90
Total	3,155.85	609.78	506.66	1,543.49	109.60	202.32	6,127.70
Medium prickly-pear group (cows 4, 5, 6):							
Corn chop	492.78	44.45	11.82	401.69	20.31	1.22	972.27
Wheat bran	363.90	85.71	29.18	228.93	17.58	15.83	741.13
Cottonseed meal	432.70	238.39	19.48	97.56	68.70	16.86	873.69
Sorghum hay	931.00	49.98	371.31	451.64	25.24	18.73	1,847.90
Prickly-pear	951.00	112.30	176.01	486.25	13.22	87.20	1,825.93
Total	3,171.38	530.83	607.80	1,666.07	145.05	139.84	6,260.92
Medium prickly-pear group (cows 8, 9):							
Corn chop	330.03	29.77	7.91	269.03	13.60	.82	651.16
Wheat bran	243.72	57.40	19.55	153.32	11.77	10.60	496.36
Cottonseed meal	289.80	159.65	13.05	65.34	46.01	11.29	585.14
Sorghum hay	512.42	27.51	204.37	248.59	13.88	10.31	1,017.08
Prickly-pear	634.89	74.98	117.51	324.62	8.83	58.21	1,219.04
Total	2,010.86	349.31	362.39	1,060.90	94.09	91.23	3,968.78
Sorghum-hay group (cows 10, 11, 12):							
Corn chop	540.79	50.74	13.22	427.09	22.30	1.32	1,055.46
Wheat bran	399.36	97.84	32.65	243.41	19.29	17.20	809.75
Cottonseed meal	474.85	272.12	21.80	103.73	75.41	18.31	966.22
Sorghum hay	1,632.02	91.14	663.56	167.05	44.24	32.49	3,230.50
Total	3,047.02	511.84	731.23	1,541.28	161.24	69.32	7,028.15

SECOND PERIOD (80 DAYS)

Feed.	Dry matter.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.	Amids.	Total.
Heavy prickly-pear group (cows 4, 5, 6):							
Corn chop	450.90	51.00	14.61	338.29	16.21	1.67	872.68
Wheat bran	336.95	96.56	23.73	199.11	17.52	15.75	689.62
Cottonseed meal	395.44	256.14	12.95	76.29	87.84	14.92	843.58
Sorghum hay	509.37	22.59	160.85	273.29	12.23	4.98	983.31
Prickly-pear	1,892.31	190.97	171.22	1,031.25	31.15	132.94	3,449.84
Total	3,584.97	617.26	383.36	1,918.23	164.95	170.26	6,839.03
Medium prickly-pear group (cows 1, 2, 3):							
Corn chop	373.67	34.37	13.26	299.75	13.74	1.36	736.15
Wheat bran	279.24	65.07	21.52	176.42	14.85	12.78	569.88
Cottonseed meal	327.72	172.62	11.74	67.59	74.45	12.11	666.23
Sorghum hay	1,104.42	39.81	381.67	633.57	27.12	10.57	2,197.16
Prickly-pear	1,021.32	83.82	101.13	595.11	17.19	70.26	1,888.83
Total	3,106.37	395.69	529.32	1,772.44	147.35	107.08	6,058.25
Medium prickly-pear group (cows 10, 11, 12):							
Corn chop	485.88	44.69	17.24	389.77	17.87	1.76	957.21
Wheat bran	363.09	84.61	27.98	229.41	19.31	16.62	741.02
Cottonseed meal	428.12	224.46	15.27	87.89	96.80	15.74	868.28
Sorghum hay	1,070.99	38.60	370.11	614.39	26.30	10.25	2,130.64
Prickly-pear	1,021.32	83.82	101.13	595.11	17.19	70.26	1,888.83
Total	3,369.40	476.18	531.73	1,916.57	177.47	114.63	6,585.98
Sorghum hay group (cows 8, 9):							
Corn chop	358.46	34.30	12.97	278.59	13.19	1.29	698.80
Wheat bran	267.88	64.93	21.04	163.97	14.25	12.14	544.21
Cottonseed meal	314.37	172.26	11.48	62.83	71.43	11.49	643.86
Sorghum hay	1,265.36	47.44	445.78	703.28	31.07	11.98	2,504.91
Total	2,206.07	318.93	491.27	1,208.67	129.94	36.90	4,391.78

TABLE XXX.—*Nutrients digested: Group totals (second year)*

FIRST PERIOD (80 DAYS)

Feed.	Nutrient.						Total.
	Dry matter.	Crude protein (N×6.25).	Crude fiber.	Nitrogen-free extract.	Ether extract.	Amids.	
Sorghum-silage group (cows 3, 14, 17, 19, 20):	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Corn chop...................	1,065.31	106.14	14.23	871.44	44.43	3.63	2,105.18
Wheat bran.................	795.34	193.09	35.39	541.43	25.60	38.55	1,629.40
Cottonseed meal............	942.89	488.89	20.03	310.52	89.92	34.27	1,886.52
Cottonseed hulls............	1,483.67	9.46	831.37	509.08	35.19	1.16	2,869.93
Sorghum silage.............	1,519.28	84.95	412.80	786.81	65.13	56.42	2,925.39
Total....................	5,806.49	882.53	1,313.82	3,019.28	260.27	134.03	11,416.42
Prickly-pear group (cows 6, 9, 15, 16, 18):							
Corn chop...................	1,042.57	103.88	13.93	852.84	43.48	3.56	2,060.26
Wheat bran.................	778.37	188.97	34.63	529.87	25.05	37.73	1,594.62
Cottonseed meal............	922.77	478.46	19.61	303.89	88.00	33.54	1,846.27
Cottonseed hulls............	1,488.26	9.49	833.94	510.66	33.57	1.16	2,877.08
Prickly-pear	1,369.63	62.21	114.69	1,001.58	17.78	20.98	2,586.87
Total....................	5,601.60	843.01	1,016.80	3,198.84	207.88	96.97	10,965.10
Prickly-pear group (cows 8, 11, 12):							
Corn chop...................	870.21	86.71	11.62	711.85	36.29	2.97	1,719.65
Wheat bran.................	649.69	157.73	28.91	442.28	20.91	31.49	1,331.01
Cottonseed meal............	770.22	399.36	16.37	253.65	73.45	27.99	1,541.04
Cottonseed hulls............	796.60	5.08	446.37	273.33	18.90	.62	1,540.90
Prickly-pear	780.43	35.44	65.35	570.71	10.13	11.96	1,474.02
Total....................	3,867.15	684.32	568.62	2,251.82	159.68	75.03	7,606.62
Cottonseed-hulls group (cows 4, 5, 10):							
Corn chop...................	881.13	87.80	11.77	720.78	36.75	3.01	1,741.24
Wheat bran.................	657.84	159.72	29.27	447.83	21.17	31.89	1,347.72
Cottonseed meal............	779.88	404.38	16.57	256.83	74.37	28.34	1,560.37
Cottonseed hulls............	1,752.91	11.18	982.23	601.46	41.59	1.37	3,390.74
Total....................	4,071.76	663.07	1,039.84	2,026.90	173.88	64.61	8,040.06

SECOND PERIOD (80 DAYS)

Feed.	Dry matter.	Crude protein (N×6.25).	Crude fiber.	Nitrogen-free extract.	Ether extract.	Amids.	Total.
Sorghum-silage group (cows 6, 9, 15, 16, 18):							
Corn chop...................	1,010.65	90.18	15.25	852.92	23.87	1.47	1,994.34
Wheat bran.................	741.27	183.99	33.89	497.65	23.99	23.34	1,504.11
Cottonseed meal............	838.89	475.73	27.56	198.92	105.66	66.58	1,713.34
Cottonseed hulls............	1,533.13	9.93	870.19	534.89	23.68	8.40	2,980.22
Sorghum silage.............	1,193.40	66.16	276.05	650.26	65.09	58.64	2,309.60
Total....................	5,317.34	825.97	1,222.94	2,734.64	242.29	158.43	10,501.66
Prickly-pear group (cows 3, 14, 17, 19, 20):							
Corn chop...................	905.50	80.80	13.66	764.19	21.39	1.32	1,786.86
Wheat bran.................	664.14	164.83	30.37	445.88	21.50	20.91	1,347.63
Cottonseed meal............	751.61	426.20	24.69	178.21	94.66	59.65	1,535.02
Cottonseed hulls............	1,357.49	8.79	770.50	473.62	20.97	7.43	2,638.80
Prickly-pear.................	1,139.78	64.86	100.56	818.02	17.34	32.16	2,172.72
Total....................	4,818.52	745.48	939.78	2,679.92	175.86	121.47	9,481.03
Prickly-pear group (cows 4, 5, 10):							
Corn chop...................	572.04	51.05	8.63	482.77	13.51	.83	1,128.83
Wheat bran.................	419.57	104.13	19.18	281.69	13.58	13.21	851.36
Cottonseed meal............	474.83	269.28	15.60	112.59	59.80	37.69	969.79
Cottonseed hulls............	722.47	4.68	410.07	252.06	11.16	3.96	1,404.40
Prickly-pear.................	650.07	37.00	57.36	466.56	9.89	18.34	1,239.22
Total....................	2,838.98	466.14	510.84	1,595.67	107.94	74.03	5,593.60
Cottonseed-hulls group (cows 8, 11, 12):							
Corn chop...................	735.77	65.66	11.10	620.94	17.38	1.07	1,451.92
Wheat bran.................	539.66	133.69	24.68	362.31	17.46	16.99	1,095.03
Cottonseed meal............	610.73	346.34	20.06	144.81	76.92	48.47	1,247.33
Cottonseed hulls............	1,627.46	10.54	923.74	567.80	25.15	8.91	3,163.60
Total....................	3,513.62	556.47	979.58	1,695.86	136.91	75.44	6,957.88

TABLE XXXI.—*Composition of feeds used in digestion trials*

Feed.	No. of digestion trial.	Water.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Corn chop................	1 to 4	12.33	1.27	9.12	2.17	71.61	3.50
Do..................	5	11.79	1.23	9.40	1.83	72.95	2.80
Wheat bran..............	1 to 4	9.77	6.70	15.38	10.05	53.02	5.08
Do..................	5	9.91	6.02	17.80	8.67	53.50	4.10
Cottonseed meal.........	1 to 4	9.53	6.22	44.93	6.82	18.50	14.00
Do..................	5	10.27	6.15	40.96	6.08	23.15	13.39
Sorghum hay.............	1 to 5	6.22	8.84	5.65	29.50	47.71	2.08
Prickly-pear (air-dried)...	1	5.87	20.60	6.47	10.94	53.77	2.35
Do..................	2	8.30	18.78	6.88	10.60	53.21	2.23
Do..................	3	9.05	16.96	4.95	13.15	54.37	1.52
Do..................	4	8.09	16.47	4.31	12.52	57.17	1.44
Do..................	5	8.24	18.24	5.27	10.04	56.07	2.14
Refused sorghum hay....	1 (cow 11)	5.58	7.95	4.07	33.16	47.91	1.33
Do..................	1 (cow 12)	5.27	15.43	6.08	28.65	41.73	2.84
Do..................	3 (cow 11)	5.51	15.02	4.26	31.95	40.48	2.78
Do..................	3 (cow 12)	5.59	8.99	3.89	31.60	47.13	2.80
Do..................	5 (cow 1)	7.90	15.61	6.53	33.19	34.75	2.02
Common salt............	1 to 5	3.37	{ Sodium chlorid, 94.75 per cent; undetermined, 1.88 per cent.					

TABLE XXXII.—*Composition of feces voided and air-dried during digestion trials*

No. of cow.	No. of digestion trial.	Water.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
11........................	1	5.82	16.12	10.37	20.20	45.16	2.33
12........................	1	5.48	20.65	9.47	17.16	45.34	1.90
1.........................	2	4.08	30.30	4.23	16.33	43.78	1.28
2.........................	2	6.24	20.62	4.38	16.23	49.59	2.94
11........................	3	8.05	20.31	8.69	17.55	42.47	2.93
12........................	3	6.93	15.25	8.07	18.72	47.87	3.16
1.........................	4	5.18	22.11	3.22	16.89	50.02	2.58
3.........................	4	5.48	29.71	4.05	19.06	39.80	1.90
1.........................	5	5.58	20.17	8.21	17.70	45.64	2.70
11........................	5	4.73	22.79	2.84	16.31	51.03	2.30

TABLE XXXIII.—*Results of digestion trial 1, cow 11, on a ration of hay and grain*

Feed.	Quantity.	Constituent.					
		Dry matter.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
	Pounds.	Pounds.	Pounds.	Pounds.	Pounds.	Pounds.	Pounds.
Corn chop.....................	22	19.2874	0.2794	2.0064	0.4774	15.7542	0.7700
Wheat bran...................	22	19.8506	1.4740	3.3836	2.2110	11.6644	1.1176
Cottonseed meal...............	22	19.9034	1.3684	9.8846	1.5004	4.0700	3.0800
Sorghum hay..................	180	168.8040	15.9120	10.1700	53.1000	85.8780	3.7440
Refused sorghum hay.........	24.2	22.8496	1.9239	.9849	8.0247	11.5942	.3219
Common salt..................	.625	.5922	.5922				
Total (less refused hay)...	....	205.5880	17.7021	24.4597	49.2641	105.7724	8.3897
Feces:							
Wet................	379.85	78.6309	13.4586	8.6579	16.8650	37.7041	1.9453
Air-dried..........	83.49	....	....	....	....	....	....
Digested.................	....	126.9571	4.2435	15.8018	32.3991	68.0683	6.4444
Digested (per cent).....	....	61.75	23.97	64.60	65.77	64.35	76.81

TABLE XXXIV.—*Results of digestion trial 1, cow 12, on a ration of hay, grain, and medium prickly-pear*

Feed.	Quantity.	Constituent.					
		Dry matter.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Corn chop....................	30	26.3010	0.3810	2.7360	0.6510	21.4830	1.0500
Wheat bran...................	30	27.0690	2.0100	4.6140	3.0150	15.9060	1.5240
Cottonseed meal..............	30	27.1410	1.8660	13.4790	2.0460	5.5500	4.2000
Prickly-pear (wet)	600	56.7603	12.4218	3.9014	6.5968	32.4233	1.4170
Prickly-pear (air-dried)	60.3	....	....	....	....	....	....
Sorghum hay..................	100	93.7800	8.8400	5.6500	29.5000	47.7100	2.0800
Refused sorghum hay..........	20	18.9460	3.0860	1.2160	5.7300	8.3460	.5680
Common salt..................	.625	.5922	.5922	....	....	....	....
Total (less refused hay)...	....	212.6975	23.0250	29.1644	36.0788	114.7263	9.7030
Feces:							
Wet...............	388.20	75.0773	16.4023	7.5220	13.6302	36.0136	1.5092
Air-dried..........	79.43	....	....	....	....	....	....
Digested..............	....	137.6202	6.6227	21.6424	22.4486	78.7127	8.1938
Digested (per cent).....	....	64.70	28.76	74.21	62.22	68.69	84.45

TABLE XXXV.—*Results of digestion trial 2, cow 1, on a ration exclusively of prickly-pear*

Feed.	Quantity.	Constituent.					
		Dry matter.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Prickly-pear (wet)..............	1,170	106.5371	21.8186	7.9932	12.3151	61.8194	2.5908
Prickly-pear (air-dried).........	116.18	....	....	....	....	....	....
Common salt..................	.625	.5922	.5922	....	....	....	....
Total.................	....	107.1293	22.4108	7.9932	12.3151	61.8194	2.5908
Feces:							
Wet...............	324.90	44.7851	14.1471	1.9750	7.6245	20.4409	.5976
Air-dried..........	46.69	....	....	....	....	....	....
Digested.................	....	62.3442	8.2637	6.0182	4.6906	41.3785	1.9932
Digested (per cent).....	....	58.20	36.87	75.29	38.09	66.93	76.93

TABLE XXXVI.—*Results of digestion trial 2, cow 2, on a ration of grain, hay, and heavy prickly-pear*

Feed.	Quantity.	Constituent.					
		Dry matter.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Corn chop	18	15.7806	0.2286	1.6416	0.3906	12.8898	0.6300
Wheat bran	18	16.2414	1.2060	2.7684	1.8090	9.5436	.9144
Cottonseed meal	18	16.2846	1.1196	8.0874	1.2276	3.3300	2.5200
Prickly-pear (wet)	1,080	....	....	....	....	....	....
Prickly-pear (air-dried)	107.24	98.3391	20.1397	7.3781	11.3674	57.0624	2.3915
Sorghum hay	45	42.2010	3.9780	2.5425	13.2750	21.4695	.9360
Common salt	.625	.5922	.5922	....	....	....	....
Total	....	189.4389	27.2641	22.4180	28.0695	104.2953	7.3919
Feces:							
Wet	423.30	....	....	....	....	....	....
Air-dried	80.47	75.4487	16.5929	3.5246	13.0603	39.9051	2.3658
Digested	....	113.9902	10.6712	18.8934	15.0093	64.3902	5.0261
Digested (per cent)	....	60.17	39.14	84.28	53.47	61.74	67.99

TABLE XXXVII.—*Digestion trial 3, cow 11, on a ration of grain, hay, and medium prickly-pear*

Feed.	Quantity.	Constituent.					
		Dry matter.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Corn chop	22	19.2874	0.2794	2.0064	0.4774	15.7542	0.7700
Wheat bran	22	19.8506	1.4740	3.3836	2.2110	11.6644	1.1176
Cottonseed meal	22	19.9034	1.3684	9.8846	1.5004	4.0700	3.0800
Prickly-pear (wet)	600	54.5154	10.1658	2.9670	7.8821	32.5894	.9111
Prickly-pear (air-dried)	59.54	....	....	....	....	....	....
Sorghum hay	100	93.7800	8.8400	5.6500	29.5000	47.7100	2.0800
Refused sorghum hay	10	9.4490	1.5020	.4260	3.1950	4.0480	.2780
Common salt	.625	.5922	.5922	....	....	....	....
Total (less refused hay)	....	198.4800	21.2178	23.4656	38.3759	107.7400	7.6807
Feces:							
Wet	361.40	....	....	....	....	....	....
Air-dried	78.17	71.8773	15.8763	6.7930	13.7188	33.1988	2.2904
Digested	....	126.6027	5.3415	16.6726	24.6571	74.5412	5.3903
Digested (per cent)	....	63.79	25.17	71.05	64.25	69.19	70.18

TABLE XXXVIII.—*Results of digestion trial 3, cow 12, on a ration of grain and hay*

Feed.	Quantity.	Constituent.					
		Dry matter.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Corn chop....................	30	26.3010	0.381	2.736	0.651	21.483	1.050
Wheat bran...................	30	27.0690	2.010	4.614	3.015	15.906	1.524
Cottonseed meal..............	30	27.1410	1.866	13.479	2.046	5.550	4.200
Sorghum hay..................	160	150.0480	14.1440	9.0400	47.200	76.3360	3.3280
Refused sorghum hay.........	15.25	14.3975	1.3710	.5932	4.8190	7.1873	.4270
Common salt..................	.625	.5922	.5922	....	....	....	....
Total (less refused hay)...	....	216.7537	17.6222	29.2758	48.0930	112.0877	9.6750
Feces:							
Wet...............	443.10	79.8820	13.0891	6.9265	16.0674	41.0868	2.7122
Air-dried..........	85.83	....	....	....	....	....	....
Digested..............	....	136.8717	4.5331	22.3493	32.0256	71.0009	6.9628
Digested (per cent).....	....	63.15	25.72	76.34	66.59	63.34	71.97

TABLE XXXIX.—*Results of digestion trial 4, cow 1, on a ration of grain, hay, and heavy prickly-pear*

Feed.	Quantity.	Constituent.					
		Dry matter.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Corn chop....................	14	12.2738	0.1778	1.2768	0.3038	10.0254	0.4900
Wheat bran...................	14	12.6322	.9380	2.1532	1.4070	7.4228	.7112
Cottonseed meal..............	14	12.6658	.8708	6.2902	.9548	2.5900	1.9600
Prickly-pear (wet)...........	1,200	121.4315	21.7602	5.6944	16.5414	75.5330	1.9025
Prickly-pear (air-dried).......	132.12	....	....	....	....	....	....
Sorghum hay..................	50	46.890	4.420	2.825	14.750	23.855	1.040
Common salt..................	.625	.5922	.5922	....	....	....	....
Total.....................	....	206.4855	28.7590	18.2396	33.9570	119.4262	6.1037
Feces:							
Wet...............	422.80	83.0243	19.3595	2.8194	14.7889	43.7975	2.2590
Air-dried..........	87.56	....	....	....	....	....	....
Digested..............	....	123.4612	9.3995	15.4202	19.1681	75.6287	3.8447
Digested (per cent).....	....	59.79	32.68	84.54	56.45	63.33	62.99

TABLE XL.—*Results of digestion trial 4, cow 3, on a ration exclusively of prickly-pear*

Feed.	Quantity.	Constituent.					
		Dry matter.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Prickly-pear (wet)...............	1,200	121.4315	21.7602	5.6944	16.5414	75.5330	1.9025
Prickly-pear (air-dried).........	132.12	....	....	....	....	....	....
Common salt..................	.625	.5922	.5922	....	....	....	....
Total.......................	....	122.0237	22.3524	5.6944	16.5414	75.5330	1.9025
Feces:							
Wet...............	301.95	42.7513	13.4378	1.8318	8.6208	18.0015	.8549
Air-dried..........	45.23	....	....	....	....	....	....
Digested...............	....	79.2724	8.9146	3.8626	7.9206	57.5315	1.0431
Digested (per cent).....	....	64.96	39.88	67.83	47.88	76.17	54.83

TABLE XLI.—*Results of digestion trial 5, cow 1, on a ration of grain, hay, and medium prickly-pear*

Feed.	Quantity.	Constituent.					
		Dry matter.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Corn chop......................	14	12.3494	0.1722	1.3160	0.2562	10.2130	0.3920
Wheat bran....................	14	12.6126	.8428	2.4920	1.2138	7.4900	.5740
Cottonseed meal...............	14	12.5622	.8610	5.7344	.8512	3.2410	1.8746
Prickly-pear (wet)..............	600	59.9559	11.9180	3.4434	6.5601	36.6361	1.3983
Prickly-pear (air-dried).........	65.34	....	....	....	....	....	....
Sorghum hay...................	100	93.7800	8.8400	5.6500	29.5000	47.7100	2.0800
Refused sorghum hay...........	3.8	3.4998	.5932	.2481	1.2612	1.3205	.0768
Salt...........................	.625	.5922	.5922	....	....	....	....
Total (less refused hay)...	....	188.3525	22.6330	18.3877	37.1201	103.9696	6.2421
Feces:							
Wet...............	453.35	....	....	....	....	....	....
Air-dried..........	82.87	78.2459	16.7149	6.8036	14.6680	37.8219	2.2375
Digested...............	....	110.1066	5.9181	11.5841	22.4521	66.1477	4.0046
Digested (per cent)......	....	58.46	26.15	63.00	60.49	63.62	64.15

TABLE XLII.—*Results of digestion trial 5, cow 11, on a ration of grain, hay, and heavy prickly-pear*

Feed.	Quantity.	Constituent.					
		Dry matter.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Corn chop....................	22	19.4062	0.2706	2.0680	0.4026	16.0490	0.6160
Wheat bran...................	22	19.8198	1.3244	3.9160	1.9074	11.7700	.9020
Cottonseed meal..............	22	19.7406	1.3530	9.0112	1.3376	5.0930	2.9458
Prickly-pear (wet)............	1,050	....	....	....	....	....	....
Prickly-pear (air-dried)........	114.34	104.9183	20.8556	6.0257	11.4797	64.1104	2.4469
Sorghum hay..................	50	46.8900	4.4200	2.8250	14.7500	23.8550	1.0400
Common salt..................	.625	.5922	.5922	....	....	....	....
Total.....................	....	211.3671	28.8158	23.8459	29.8773	120.8774	7.9507
Feces:							
Wet................	393.05	....	....	....	....	....	....
Air-dried..........	86.35	82.2656	19.6792	2.4523	14.0837	44.0644	1.9860
Digested...............	....	129.1015	9.1366	21.3936	15.7936	76.8130	5.9647
Digested (per cent).....	....	61.08	31.71	89.72	52.86	63.55	75.02

PLATE F

Opuntia cyanella, one of the principal species of prickly-pear used in these experiments.

Fig. 1.—Mature joint; ½ natural size on a photographic background.

Fig. 2.—A flower bud; natural size.

Fig. 3.—Young joint showing rudimentary leaves.

Fig. 4.—Flower; ½ natural size.

Fig. 5.—A fruit; natural size.

PLATE LXI

Fig. 1.—Singeing prickly-pear with a gasoline torch.

Fig. 2.—Cutting prickly-pear with a hoe, the blade of which is bent so as to be in line with the handle.

A NASTURTIUM WILT CAUSED BY BACTERIUM SOLANACEARUM

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INTRODUCTION

On July 21, 1914, some badly wilted nasturtiums (*Tropaeolum majus*) were received from Dr. John Arthur Luetscher of Baltimore, Md., who wrote concerning them:

Seven years ago I raised a fine lot of nasturtiums, but in the last six years I have hardly been able to get a blossom, although the plants have been in the same soil and several times in the same plat. The leaves wither and the plant dies.

The plants, which were of the dwarf variety and much-branched, were poorly developed, and the leaves mostly wilted, yellowed, or dead (Pl. LXIII). The stems had a peculiar translucent or water-soaked appearance, allowing the vascular bundles to show as darkened streaks beneath the unbroken epidermis. When the stem was cut across, there oozed from these bundles a grayish white viscid slime which became brown on standing.

ISOLATION OF THE ORGANISM

On cross-sectioning such stems the vessels were found to be clogged with bacteria, often every bundle being entirely occluded. Agar-poured plates gave pure cultures of a white bacterial organism. Inoculations made from colonies on these plates into nasturtium stems produced signs of the disease—i. e., wilted leaves and water-soaked stems—within seven days (Pl. LXIV). From one of these stems the organism was reisolated on agar-poured plates and again produced typical wilt within four days when inoculated into healthy young nasturtiums, using sub-cultures from single colonies.

NATURE OF THE ORGANISM

Cultural work was then begun, but it was not until the growth on potato cylinders began to blacken that the identity of the organism with *Bacterium solanacearum*[1] was suspected. To test this hypothesis, inoculations were at once made into tomatoes (*Lycopersicon esculentum*), the only available plants being rather old. The result was the formation of numerous adventive roots in the vicinity of the needle pricks and the slow wilt of a few leaflets. Vessels were browned and filled with these

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[1] Originally described by Erwin F. Smith as *Bacillus solanacearum* under the supposition that it was peritrichiate, but afterwards transferred by him to the genus Bacterium, in accordance with his system of nomenclature *in his* Bacteria in Relation to Plant Diseases. v. 1, p. 171. Washington, D. C., 1905. (Carnegie Inst. Wash. Pub. 27.)

bacteria, as shown by microscopic examination and by poured plates. The plants then outgrew the disease. While not conclusive, these results did not contradict my supposition, since the organism plated from tobacco (*Nicotiana* spp.) and tomato often gives no more marked results when inoculated into old plants.

FURTHER CROSS-INOCULATIONS

A virulent strain of *B. solanacearum* obtained from tobacco from Creedmore, N. C., during the summer of 1914 was then available for comparison, and inoculations were made with this into nasturtiums of the tall variety by means of needle pricks from young agar subcultures. After 10 days all plants showed one or more wilted leaves and an abundance of the characteristic adventive roots near the point of inoculation (Pl. LXV, fig. 1). A month later one of these stems had produced adventive roots at intervals from 7 inches above the pricks to 20 inches below them, and in one case where the stem hung near the ground they were 3 inches long and had taken hold in the soil. Bacteria were present the entire length of the stem, which was now entirely leafless. Inoculations into dwarf nasturtiums produced a more rapid wilt but no adventive roots.

On young tobacco, prick inoculations with the nasturtium organism caused in five days an internal dark streak (visible on the surface) running several inches up and down the stem from the point of inoculation and the wilt of one or two leaves, but the plants always recovered. Check pricks produced no effect.

Inoculations with the nasturtium organism into *very young* tomato plants resulted in the rapid and complete wilt of the plants (Pl. LXVI, fig. 1). The entire vascular system became gorged with bacteria. Poured plates gave pure cultures of *Bacterium solanacearum*, as determined by cultures on typical media and by successful reinoculations into both tomato and tobacco plants.

TESTS ON OTHER PLANTS

A variety of plants were tested for susceptibility. Prick inoculations were made with both the Creedmore tobacco organism and the nasturtium organism into pelargoniums, soy beans (*Glycine hispida*), and lettuce (*Lactuca sativa*), all with negative results. Owing to the fact that Honing[1] in Sumatra has reported this disease on several composites and in young teak trees (Verbenaceae), inoculations were made on hothouse ageratum and on common cultivated verbena. Both became diseased but rather slowly. After 10 days the ageratum showed distortion of the leaves, one half being paler and smaller than the other, and after

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[1] Honing, J. A., Een geval van slijmziekte in de djattibibit. Meded. Deli Proefstat. te Medan, Jrg. 7, Afl. 1, p. 12–15, also Naschrift, p. 59, 1912. See review in Smith, Erwin F., Bacteria in relation to plant diseases, v. 3, p. 254.

15 days complete wilt of several leaves occurred. The results were checked under the microscope. For the most part the plants outgrew the disease. Verbenas showed wilt within two weeks, and after three weeks the tips, as well as the leaves, for 2 inches below the point of inoculation, were completely wilted. Agar-poured plates from one of these stems gave pure cultures of *B. solanacearum.*

NATURAL METHODS OF INFECTION

The organism may enter the nasturtium plant through wounded roots or shoots or through the stomata. To demonstrate root infection, six nasturtium seeds were planted in each of four pots. Two pots were watered with a suspension from young agar cultures of the nasturtium organism and then covered with fresh soil. The others were held as checks. When the plants had four good leaves, the soil was worked in all the pots deeply enough to break some roots. Six weeks later one plant in an inoculated pot was badly wilted, and 10 days later four others succumbed, while those in the check pots were perfectly healthy. One week later all but 3 of the 12 plants in the inoculated pots had succumbed. Sections from stems of a number of these wilting plants were examined under the microscope. The vessels were seen to be clogged with bacteria, and there was the usual tissue disorganization.

Before the organism was identified, spray experiments were started to determine whether stomatal infections could be obtained. Well-grown plants of both tall and dwarf varieties of nasturtiums were sprayed in cages with a suspension from 3-day agar-slant cultures. Repeated spraying with sterile water kept the plants moist for 30 hours, after which they were removed from the cages. Six days later a few minute brownish spots appeared on the leaves, but these did not enlarge materially. Four weeks later, however, one plant was characteristically wilted, and within three weeks from this time all of the dwarf plants and one of the tall ones had succumbed, with characteristic bacterial infection of the vascular system.

Another experiment was made with young plants, each bearing two large leaves. They were sprayed in cages with suspensions from young agar cultures and kept moist for 48 hours. Four days after the experiment was started the large leaves all showed decided brown spotting and water-soaked areas. The spots centered about the stomata in every case examined, and often, but not always, they were marginal. After two weeks the spots had coalesced in many places and appeared to be affecting the small veins of the blade. Poured plates from such spots gave typical colonies of *B. solanacearum.* Portions of the leaves in both these stages were embedded, sectioned, and stained, and bacterial foci found in the substomatic chamber, thus demonstrating stomatal infection. In the younger stages the bacteria appear in the stomatal opening, as well as in the large chamber beneath (fig. 1). In older stages the

collapsed parenchyma is evidence of their presence, and careful search finds them lying closely appressed to the cell walls when they are not abundant in the intercellular spaces. In this stage they are also in the neighboring vessels of the leaf (fig. 2). In stained sections the walls of the vessels often show injury by taking a deeper stain than normal ones, even when the bacteria do not appear to have penetrated to their interior.

One leaf, which showed browning of some of the smaller veins, was sectioned at various points on the veins and petiole. The bacteria were numerous in the vessels of the browned area and for some distance below, but thinned out downward so that none were found in the base of the petiole or in the stem of the plant. In another case the bacteria
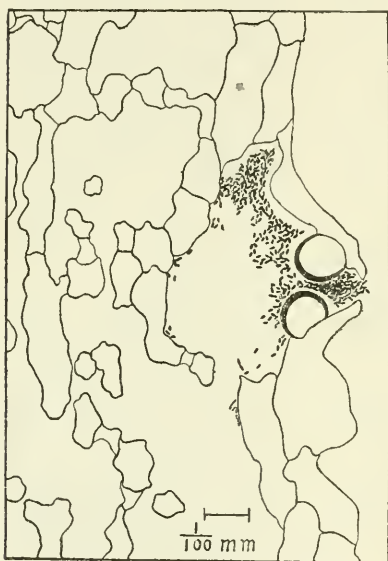
Fig. 1.—Section of nasturtium leaf four days after spraying with suspension of *Bacterium solanacearum*. Serially adjacent sections show bacteria throughout the substomatic chamber.
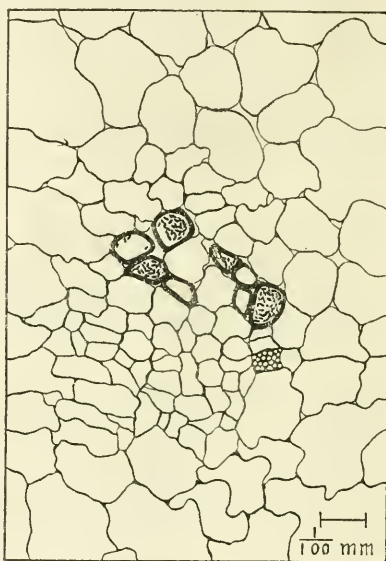
Fig. 2.—Cross section of a vein of nasturtium leaf, showing vascular infection nine days after spraying with suspension of *Bacterium solanacearum*.

were traced in the vessels all the way from the wilting leaf blade to the stem of the plant. Three plants in this set finally wilted completely. An early stage of vascular occlusion and cavity formation in the stem of a nasturtium, like that shown in Plate LXIII, is illustrated in figure 3.

Several attempts to produce stomatal infection on tomatoes and tobacco were made, but without success.

SUSCEPTIBILITY OF THE NASTURTIUM

From comparative needle-prick inoculations on nasturtium, tomato, and tobacco with the Creedmore tobacco organism, which was beginning to lose its virulence, it would appear that the nasturtium is very susceptible to infection by *B. solanacearum*, since it wilted readily, while

tobacco and tomato, except when very young, wilted only slightly and recovered quickly. The converse of this experiment led to the same conclusion—i. e., that the nasturtium is more susceptible than the tomato or tobacco—because the organism isolated from the nasturtium was more infectious to the nasturtium than to tobacco or tomato. Possibly the susceptibility of nasturtium may be due to the great succulence of the nasturtium stems. The Medan (Sumatra) tobacco organism,
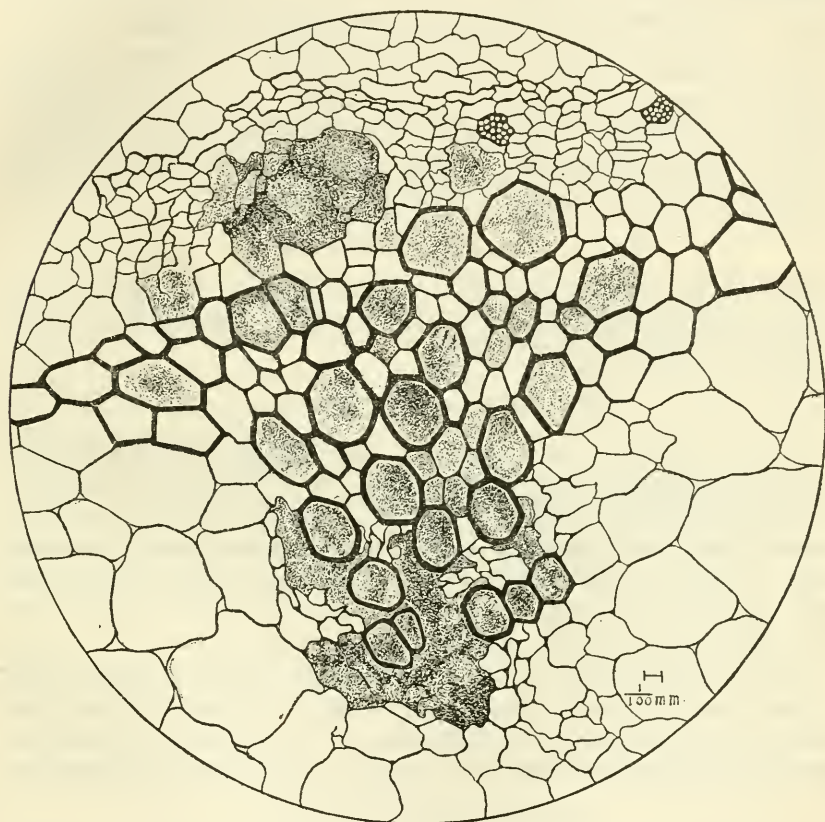
FIG. 3.—Cross section of stem of one of the infected nasturtiums from Baltimore, Md., showing the bacterial invasion of a bundle with the beginning of bacterial cavities. Two sieve plates are visible in the center phloem.

which had been extremely virulent but had been on media for a longer time than the Creedmore organism, was not able to infect either nasturtiums or tomatoes.

EFFECT ON THE TISSUES

The more tender parts of badly diseased plants become so translucent that the occluded browned vessels may be seen clearly through the water-soaked but unshriveled parenchyma (Pl. LXVI, fig. 3). In other cases the course of the affected bundle or bundles is marked superfi-

cially by sunken, reddish brown streaks or patches (Pl. LXV, fig. 2). Generally in the case of prick inoculations on nasturtiums of the tall variety adventive roots are formed at various points on the stem. These remain rudimentary except where the stems are near the ground, when they may become functioning roots. Check pricks failed to produce any root formation. No adventive roots occurred on any of the inoculated plants of the dwarf variety.

MORPHOLOGY OF THE NASTURTIUM ORGANISM

The organism is a short rod with rounded ends, 0.6 by 0.8 to 1.3μ, motile by means of one to three polar flagella. No spores or capsules occur on any media. Chains of 10 to 15 individuals are formed in 0.5 and 1 per cent salt bouillon. Similar chains are formed in 0.5 per cent salt bouillon by the Creedmore tobacco organism.

STAINING REACTIONS

With carbol fuchsin polar staining is obtained. The organism does not stain by Gram's method and is not acid-fast. Flagella were demonstrated by Löwitt's flagella stain.

CULTURAL CHARACTERS

In all the cultural tests made with this organism it agrees substantially with *Bacterium solanacearum* Erw. Sm. Growth was studied in the following media: Agar plates, slants, and stabs; gelatin plates and stabs; potato cylinders; beef bouillon; fermentation tubes containing water + 1 per cent Witte's peptone + 1 per cent dextrose, saccharose, lactose, maltose, mannit, or glycerin; milk; litmus milk; Cohn's solution; Uschinsky's solution; and Fermi's solution.

Growth is retarded by 0.5 per cent of sodium chlorid in beef bouillon; is prevented by 2 per cent, and is very weak in 1 per cent. This is true also for the Creedmore tobacco organism, which was used for comparison. No record has been previously given for *B. solanacearum* in this medium.

TEMPERATURE RELATIONS

The optimum for growth is about 30° C. No growth occurs at 39° C., very weak growth at 12° C., and none at 10° C. The thermal death-point lies between 48° and 52° C.

DESICCATION OF THE ORGANISM

When dried on sterile covers from young peptone-beef-bouillon cultures and kept in the dark at room temperature (21° C.), most covers gave growth after 24 hours when dropped into suitable bouillon, very few after 2 days', and none after 3 days' drying. The bacteria from 24-hour bouillon cultures were more sensitive to drying than those from 8-day-old cultures.

SUMMARY

The nasturtium is subject to a bacterial wilt disease, observed for the first time in the summer of 1914, which prevents blossoming, stunts the plants, and finally kills them. It is caused by a bacterium that in all morphological, cultural, and infectious characters agrees with *Bacterium solanacearum* Erw. Sm.

Cross-inoculations on the tomato and tobacco produced successful and typical wilt of these plants, while inoculations on the nasturtium with a virulent strain of *B. solanacearum*, isolated from tobacco, gave typical nasturtium wilt.

Infection takes place from infected soil through broken roots, but stomatal infection has also been demonstrated.

Cultivated ageratums and verbenas were found susceptible to infection with both the nasturtium and the Creedmore (N. C.) tobacco strains of *B. solanacearum*.

This paper adds another family to those already known to be subject to *B. solanacearum*. Described from the tomato, the potato, and the eggplant in 1896 by Dr. Erwin F. Smith,[1] this organism has now been proved infectious to one or more species of each of the following families: Solanaceae, Compositae, Leguminosae, Verbenaceae, Euphorbiaceae, Bignonaceae, and Geraniaceae.

If tomatoes, eggplants, peppers, potatoes, peanuts, or tobacco have shown this wilt disease, they should not be followed by nasturtiums.

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[1] Smith, Erwin F. A bacterial disease of the tomato, eggplant, and Irish potato (Bacillus solanacearum, n. sp.). U. S. Dept. Agr., Div. Veg. Physiol. and Path. Bul. 12, 28 p., 2 pl. (1 col.). 1896.

PLATE LXIV

Nasturtium plants four days after inoculation by needle pricks on the stem, using a pure culture of the bacteria cultivated from a plant infected like that shown in Plate LXIII.

Plate LXVI

PLATE LXVI

Fig. 1.—Young tomato plants six days after inoculation by needle pricks with the nasturtium organism, *Bacterium solanacearum*.

Fig. 2.—Normal nasturtium stem enlarged to show uniform (unstriped) appearance.

Fig. 3.—A nasturtium stem inoculated with *Bacterium solanacearum*, showing striping due to bacterial invasion of the bundles.

PHOSPHORUS METABOLISM OF LAMBS FED A RATION OF ALFALFA HAY, CORN, AND LINSEED MEAL[1]

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INTRODUCTION

The ultimate object of the investigations of which this article is a partial report was to determine the influence of different quantities of protein upon the nutrition of young growing lambs. The differences in the amounts of protein consumed were secured by varying the proportions of corn and linseed meal in the rations. Such differences in the quantities of protein were therefore necessarily accompanied by corresponding differences in the quantities of phosphorus ingested by the lambs. The experimental data relating to the phosphorus metabolism of the lambs when weighing, on the average, 115 pounds are given in this paper. For convenience, the original designation of the lots as "low-protein," "medium-protein," and "high-protein" is retained.

As to the relative availability of organic and inorganic phosphorus, there is a wide difference of opinion among investigators. With regard to lambs, there is some definite evidence of the assimilation of inorganic phosphates of calcium in the work reported by Köhler and his associates.[2]

The question of the form in which the phosphorus is excreted is a matter of interest, whatever the form of ingestion. Elimination in any of the organic forms in which it was ingested would probably mean lack of assimilation or incomplete use after assimilation. Giacosa[3] says that all absorbed phytin is split with the formation of phosphates, part of the phosphates appearing in the urine and part in the feces. Bergmann[4] reports that the subcutaneous injection of glycerophosphate in lambs was followed by complete elimination as inorganic phosphates.

It is usually said that in herbivora phosphorus is eliminated almost entirely by way of the intestine. Bergmann[4] found complete elimi-

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[2] Köhler, A., Honcamp, F., Just, M., et al. Über die Assimilation des Kalkes und der Phosphorsäure aus verschiedenen Kalkphosphaten durch wachsende Tiere. *In* Landw. Vers. Sta., Bd. 61, Heft 5/6, p. 451–479. 1905.

——— ——— and Eisenkolbe, P. Weitere Untersuchungen über die Assimilation der Phosphorsäure und des Kalkes aus Kalkphosphaten durch wachsende Tiere. *In* Landw. Vers. Stat., Bd. 65, Heft 5/6, p. 349–380. 1907.

[3] Giacosa, P. Sulla fitina (sale calcico-magnesiaco dell' acido anidrossimetilendifosforico) e suo comportarsi nell' organismo. *In* Gior. R. Accad. Med. Torino, ann. 67, no. 7/8, p. 414–416. 1904.

[4] Bergmann, W. Ueber die Ausscheidung der Phosphorsäure beim Fleisch- und Pflanzenfresser. *In* Arch. Exp. Path. u. Pharmakol., Bd. 47, Heft 1/2, p. 77–81. 1901.

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nation by this path in lambs after subcutaneous injection of either inorganic phosphates or glycerophosphates. However, the observations of Le Clerc and Cook[1] on rabbits seem to form an exception to this general rule.

The utilization and final fate of phosphorus compounds is probably in part determined by the amount and nature of the accompanying food constituents. That the amount of phosphorus in the ration affects the use made of protein by growing pigs is indicated by the work of Hart, McCollum, and Fuller.[2] Work done in this laboratory by Williams and Emmett[3] does not show a variation in the percentage or the distribution of phosphorus in the bodies or the parts of the bodies of growing pigs resulting from variations in the amount of protein consumed.

DESCRIPTION OF THE METABOLISM EXPERIMENT

ANIMALS AND RATIONS USED

Six high-grade Shropshire lambs were used in this experiment, which was a metabolism test of 12 successive days. Two representative lambs 9 months old were chosen from each of three lots which had been fed from the time of weaning, June 25, until this experiment began, December 23, on the same feeds, though the proportions fed were different. The rations of the three lots, both before and during the metabolism test, consisted of alfalfa hay, shelled corn, and old-process linseed meal. The quantity of alfalfa hay depended upon the appetite of the individual. Until December 3 of the main feeding experiment there were fed 1.5 pounds of concentrates for each 100 pounds of live weight, after which time actual increase of concentrates with the increase of live weight was deemed unwise. The daily allowance of concentrates, then, remained constant during the metabolism test. The concentrates of the ration for the low-protein lot consisted of 95 per cent of shelled corn and 5 per cent of linseed meal; for the medium-protein lot the concentrates consisted of 75 per cent of corn and 25 per cent of linseed meal; and for the high-protein lot they consisted of equal parts of corn and linseed meal. Water was accessible at all times. The amounts of feed and water consumed were determined by the difference between the quantities offered and those refused.

CARE OF ANIMALS

On December 17, 1910, each lamb was put into a metabolism cage which was large enough to allow the animal to turn around easily. To each lamb was strapped a canvas bag in which the feces were collected.

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[1] Le Clerc, J. A., and Cook, F. C. Metabolism experiments with organic and inorganic phosphorus. *In* Jour. Biol. Chem., v. 2, no. 3, p. 203–216. 1906.

[2] Hart, E. B., McCollum, E. V., and Fuller, J. G. The rôle of inorganic phosphorus in the nutrition of animals. Wis. Agr. Exp. Sta. Research Bul. 1, 38 p., 7 fig. 1909.

[3] Williams, R. H., and Emmett, A. D. A study of the phosphorus content of growing pigs with special reference to the influence of the quantity of protein consumed. Ill. Agr. Exp. Sta. Bul. 171, p. 205–230, 5 fig. 1914.

The bag was lined with oilcloth in order to prevent loss of moisture and solid material. The feces were taken from these bags at 2 p. m. daily. Under the wire grating of each cage there was a galvanized-iron tray, which acted as a collecting funnel for the urine. The lambs were fed twice each day, at 7 a. m. and at 4 p. m. The concentrates were fed about 20 minutes before the alfalfa hay, and the grain orts were collected just before the hay was fed. The hay orts of the previous feeding were collected before each new feeding. The metabolism test proper extended from December 23 to January 3, inclusive.

METHODS OF ANALYSIS

The methods of analysis used in this experiment were essentially the same as the official methods of analysis of the Association of Official Agricultural Chemists [1] for all determinations except for the different forms of phosphorus.

The methods used in determining the different forms of phosphorus in the feeds and feces were as follows:

(*a*) METHOD OF MAKING A 0.2 PER CENT HYDROCHLORIC-ACID EXTRACT.—A sample of suitable size (about 100 gm. of feces or 50 gm. of feed) was divided about equally between two 500 c. c. centrifuge bottles. A little powdered thymol and 300 c. c. of a 0.2 per cent hydrochloric-acid solution were added to each bottle. The bottles were wired spoke-wise to a bicycle wheel, which was revolved at the rate of about 38 revolutions per minute, and were shaken in this manner for from 12 to 14 hours.

The bottles were then opened, the sides of each washed from a pipette with 25 c. c. of the acid solution, and then they were placed in the centrifuge, running at the rate of about 1,700 revolutions per minute, for 10 minutes. The clear solution in each bottle was carefully decanted into a 3-liter measuring flask. Then 100 c. c. of the 0.2 per cent acid solution were added to each bottle. The bottles were shaken till the contents were homogeneous and the sides washed again with 25 c. c. of the solution. This process of extraction was repeated nine times. Generally a tenth extraction was made and tested qualitatively. The solution in the measuring flask was then made up to the mark with the 0.2 per cent hydrochloric-acid solution and thoroughly mixed. If the solution in the flask was quite thick with sediment, it was allowed to settle and then the liquid was decanted through a 10-inch qualitative filter. The filtering was repeated till the filtrate was perfectly clear.

(*b*) TOTAL ACID-SOLUBLE PHOSPHORUS DETERMINATION.—Triplicate samples of 100 c. c. each of the clear 0.2 per cent hydrochloric-acid extract were evaporated to dryness in weighed, ignited 3-inch porcelain dishes, and ashed. The total phosphorus of this ash was determined in the usual manner.

(*c*) ACID-INSOLUBLE PHOSPHORUS was determined by subtracting the total acid-soluble phosphorus from the total phosphorus of the feeds or feces.

(*d*) INORGANIC ACID-SOLUBLE PHOSPHORUS DETERMINATION.—Triplicate samples of 150 c. c. of the acid extract were each treated with 25 c. c. of magnesia mixture. This magnesia mixture was added slowly, drop by drop, while the solution was being stirred. After the solutions had stood for 15 minutes, 20 c. c. of ammonia (sp. gr. 0.90) were added to each beaker and the solutions allowed to stand for 12 hours. At the end of this time the solutions were filtered through double 11 cm. filters and the

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[1] Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 11 fig. 1908.

beakers and precipitates washed a number of times with a 2½ per cent ammonium-hydroxid solution. The inside filters and precipitates were returned to their respective beakers, treated with 25 c. c. of dilute nitric acid, and the filters well shredded with the stirring rods. Clean beakers were put under the funnels, the solutions filtered through their respective funnels, and the filters washed repeatedly with boiling distilled water. To each solution 15 c. c. of ammonia (sp. gr. 0.90) were added. The solutions were then made slightly acid with pure nitric acid. The phosphorus was precipitated with acid ammonium molybdate and the phosphorus determination continued as usual.

(*e*) Organic acid-soluble phosphorus was determined by subtracting the inorganic acid-soluble phosphorus from the total acid-soluble phosphorus.

It should be said in this connection that since these different forms of phosphorus were determined in connection with this investigation it has been clearly demonstrated in this and other laboratories that the above methods, or, in fact, any other methods at present known for the separation and estimation of inorganic and organic phosphorus in vegetable substances, are not strictly accurate and give only approximate results. It is the opinion of the authors that the above methods are as accurate for this purpose as any known at present and that the results obtained are probably a fair approximation to the true values for the inorganic and organic phosphorus in the materials examined.

LIVE WEIGHTS AND TOTAL GAINS IN BODY WEIGHT

The lambs were weighed the three days immediately before they were put into the digestion crates and the three days immediately following the metabolism test. The average of each of these three weights is given in Table I, together with the total gain in weight of each lamb from December 15 to January 5.

TABLE I.—*Live weights and total gains in live weights (in pounds) of lambs in metabolism test*

Item.	Low-protein ration.		Medium-protein ration.		High-protein ration.	
	Lamb No. 468.	Lamb No. 463.	Lamb No. 462.	Lamb No. 458.	Lamb No. 453.	Lamb No. 451.
Live weight Jan. 5............	110. 0	106. 5	118. 0	131. 0	114. 5	118. 5
Live weight Dec. 15...........	108. 5	102. 5	115. 0	125. 5	112. 0	115. 5
Total gain...............	1. 5	4. 0	3. 0	5. 5	2. 5	3. 0

WEIGHTS AND COMPOSITION OF FEEDS CONSUMED

The quantities of the feeds consumed by the individual lambs during the 12 days of the metabolism test, expressed in grams per day, are given in Table II. Lamb 463 ingested a ration relatively very high in roughage, while lambs 468 and 462 ingested a ration relatively low in roughage. Lamb 458 ingested the largest quantity of total feed. The chemical composition of the feeds consumed is given in Table III.

TABLE II.—*Rations consumed by lambs in metabolism test*

[Results expressed in grams per day]

Ration.	Lamb No.	Hay consumed.	Concentrates consumed.	Ratio of roughage to concentrates.
Low-protein....................	468	494. 9	625. 9	1 : 1. 26
	463	759. 9	556. 5	1 : 0. 73
Average.....................		627. 4	591. 2	1 : 0. 94
Medium-protein.................	462	593. 8	758. 5	1 : 1. 28
	458	793. 7	820. 0	1 : 1. 03
Average.....................		693. 8	789. 3	1 : 1. 14
High-protein...................	453	700. 5	735. 0	1 : 1. 05
	451	694. 1	747. 8	1 : 1. 08
Average.....................		697. 3	741. 4	1 : 1. 06

TABLE III.—*Chemical composition (in percentage) of feeds consumed by lambs*

Feed.	Dry matter.	Protein.[a]	Nonprotein.[b]	Ether extract.	Carbohydrates.	Ash.	Phosphorus.	Total nitrogen.
Alfalfa hay......	85. 18	12. 50	2. 16	1. 78	60. 60	8. 14	0. 216	2. 46
Corn............	88. 56	6. 89	0. 93	4. 05	75. 46	1. 23	. 269	1. 30
Linseed meal....	90. 06	31. 79	[d] 3. 16	6. 38	43. 04	5. 32	. 881	5. 83
Do.[c]........	89. 46	30. 88	[d] 3. 14	6. 36	43. 29	5. 41	. 864	5. 69

[a] Protein nitrogen × 6.25.
[b] Nonprotein nitrogen × 4.7.
[c] This sample was fed during period 3.
[d] Calculated from Armsby's data.

NUTRIENTS DIGESTED DURING THE METABOLISM TEST

The apparent coefficients of digestibility of the nutrients and the amounts of nutrients apparently digested during the metabolism test were calculated from the weights and the analyses of the feeds, orts, and feces. The weights and composition of the feces are given in Tables IV and V, respectively. The apparent coefficients of digestibility of the nutrients are given in Table VI. The quantity of dry matter, protein, nonprotein, ether extract, carbohydrates, ash, phosphorus, and nitrogen apparently digested daily by each lamb during the 12 days of the metabolism test, together with the calculated values for the metabolizable energy, or fuel values expressed in Calories, are given in Table VII.

TABLE IV.—*Weights of feces and urine of lambs*

[Results expressed in grams per day]

Material.	Low-protein ration.		Medium-protein ration.		High-protein ration.	
	Lamb No. 468.	Lamb No. 463.	Lamb No. 462.	Lamb No. 458.	Lamb No. 453.	Lamb No. 451.
Feces............	639	974	879	1, 322	983	1, 021
Urine...........	486	663	896	813	1, 263	1, 167

TABLE V.—*Chemical composition (in percentage) of the feces of lambs*

Ration.	Lamb No.	Dry matter.	Crude protein.	Ether extract.	Carbohy-drates.	Ash.	Phos-phorus.	Total nitrogen.	Meta-bolic ni-trogen.
Low-protein	468	32. 31	6. 80	1. 90	19. 77	4. 13	0. 401	1. 088	0. 567
	463	32. 65	6. 26	1. 90	20. 85	3. 93	. 332	1. 001	. 557
Average	....	32. 48	6. 53	1. 90	20. 31	4. 03	. 367	1. 045	. 562
Medium-protein....	462	32. 95	6. 30	1. 91	20. 71	4. 30	. 416	1. 007	. 504
	458	27. 64	5. 50	1. 20	17. 67	3. 46	. 342	. 880	. 432
Average	....	30. 30	5. 90	1. 56	19. 19	3. 88	. 379	. 944	. 468
High-protein.......	453	35. 55	6. 00	1. 59	20. 73	4. 43	. 520	. 960	. 422
	451	31. 68	6. 05	1. 50	19. 99	4. 32	. 522	. 969	. 438
Average	....	33. 62	6. 03	1. 55	20. 36	4. 38	. 521	. 964	. 430

TABLE VI.—*Apparent coefficients of digestibility of the nutrients of the rations of lambs*

Constituent.	Low-protein ration.			Medium-protein ration.			High-protein ration.		
	Lamb No. 468.	Lamb No. 463.	Aver-age.	Lamb No. 462.	Lamb No. 458.	Aver-age.	Lamb No. 453.	Lamb No. 451.	Aver-age.
Dry matter............	78. 9	72. 1	75. 5	75. 5	74. 0	74. 8	74. 5	74. 3	74. 4
Protein................	61. 3	56. 6	59. 0	68. 2	63. 9	66. 1	73. 7	73. 1	73. 4
Nonprotein............	100. 0	100. 0	100. 0	100. 0	100. 0	100. 0	100. 0	100. 0	100. 0
Ether extract..........	65. 0	50. 1	57. 6	63. 2	69. 0	66. 1	68. 8	70. 2	69. 5
Carbohydrates........	83. 5	76. 7	80. 1	79. 1	77. 5	78. 3	76. 5	76. 4	76. 5
Ash...................	45. 6	44. 6	45. 1	42. 0	43. 6	42. 8	45. 2	45. 3	45. 3
Phosphorus............	12. 9	2. 5	7. 7	18. 5	11. 5	15. 0	10. 0	8. 0	9. 0
Nitrogen..............	67. 3	64. 1	65. 7	73. 3	69. 9	71. 6	77. 9	77. 3	77. 6

TABLE VII.—*Amounts of nutrients apparently digested by the lambs and the metaboli-zable energy of the digested nutrients*

Constituent.	Low-protein ration.			Medium-protein ration.			High-protein ration.		
	Lamb No. 468.	Lamb No. 463.	Aver-age.	Lamb No. 462.	Lamb No. 458.	Aver-age.	Lamb No. 453.	Lamb No. 451.	Aver-age.
Dry matter.....gm..	769. 78	823. 58	796. 68	890. 62	1, 039. 30	964. 96	932. 36	934. 75	933. 56
Protein........gm..	71. 91	79. 34	75. 63	119. 05	128. 90	123. 98	165. 23	169. 15	167. 19
Nonprotein.....gm..	16. 86	22. 05	19. 46	24. 38	29. 43	26. 91	30. 65	30. 91	30. 78
Ether extract...gm..	22. 62	18. 22	20. 42	28. 78	35. 39	32. 09	34. 53	35. 91	35. 22
Carbohydrates..gm..	637. 24	670. 04	653. 64	663. 10	806. 63	734. 87	662. 78	660. 09	661. 44
Ash............gm..	22. 21	30. 85	26. 53	27. 36	35. 58	31. 47	35. 91	36. 59	36. 25
Phosphorus.....gm..	. 38	. 10	. 24	. 83	. 59	. 71	. 57	. 47	. 52
Nitrogen.......gm..	14. 37	17. 42	15. 90	24. 38	27. 04	25. 71	33. 23	33. 75	33. 49
Metabolizable en-ergy[a]....Calories..	3, 239	3, 390	3, 315	3, 626	4, 348	3, 987	3, 891	3, 909	3, 900

[a] The metabolizable energy of a ration is the energy that can be liberated and utilized in the animal body, or the gross energy less the energy contained in the feces, urine, and intestinal gases. The metabolizable energy of the rations has been calculated by multiplying the weights of the digestible nutrients by the following factors: Digestible proteins and nonproteins, 4.1; digestible carbohydrates, 4.2; and digestible ether extract, 8.8.

PHOSPHORUS OF THE RATIONS

PHOSPHORUS CONTENT OF THE FEEDS OFFERED

The percentages of the different forms of phosphorus found in the different feeds of the rations are stated in Table VIII.

TABLE VIII.—*Different forms of phosphorus in the feeds of lambs*

[Results expressed in percentage of the fresh substance]

Feed.	Total phosphorus.	Acid-insoluble phosphorus.	Acid-soluble phosphorus.		
			Total.	Inorganic.	Organic.
Alfalfa hay................	0. 216	0. 058	0. 158	0. 149	0. 009
Corn......................	. 269	. 133	. 136	. 031	. 105
Linseed meal..............	. 881	. 652	. 229	. 127	. 102

It is significant that, while the corn had 1.25 times as much total phosphorus as the alfalfa hay, the linseed meal had 4.07 times as much as the hay; also that the corn had 2.30 times as much acid-insoluble phosphorus as the hay, and the linseed meal had 11.23 times as much. The linseed meal had a much higher content of total phosphorus than either the hay or the corn, and this increased phosphorus was largely in the acid-insoluble form. Neither the corn nor the linseed meal had as high content of inorganic acid-soluble phosphorus as the hay.

Since the gradations in protein given to the three lots were made by increasing the amount of linseed meal in relation to corn and since the linseed meal was richer than the corn in total phosphorus, in acid-insoluble phosphorus, and in organic acid-soluble phosphorus, while the two concentrates were practically identical in organic acid-soluble phosphorus, it is evident that the high-protein lot received concentrates which were richer in all forms of phosphorus with the exception of the organic acid-soluble form than were those received by the medium-protein lot. The same was true of the medium-protein lot relative to the low-protein lot, but the three lots were offered practically the same quantities of organic acid-soluble phosphorus.

AMOUNTS OF PHOSPHORUS INGESTED

The average daily quantities of the different forms of phosphorus ingested by each lamb as calculated from the weights and analyses of the feeds and the orts are given in Table IX.

TABLE IX.—*Average daily amounts of the different forms of phosphorus ingested by lambs*

[Results expressed in grams per day]

Ration.	Lamb No.	Total phos- phorus.	Acid- insol- uble phos- phorus.	Acid-soluble phosphorus.		
				Total.	Inor- ganic.	Organic.
Low-protein.................{	468	2. 94	1. 24	1. 70	0. 99	0. 71
	463	3. 34	1. 31	2. 03	1. 34	. 69
Average....................	....	3. 14	1. 27	1. 87	1. 17	. 70
Medium-protein............{	462	4. 48	2. 29	2. 19	1. 34	. 85
	458	5. 11	2. 58	2. 53	1. 61	. 92
Average....................	....	4. 80	2. 44	2. 36	1. 47	. 89
High-protein...............{	453	5. 68	3. 25	2. 43	1. 61	. 82
	451	5. 79	3. 31	2. 48	1. 64	. 84
Average....................	....	5. 74	3. 28	2. 46	1. 63	. 83

The low values for total and for inorganic acid-soluble phosphorus with lamb 468 are to be accounted for by his relatively low consumption of hay.

The lot differences in the total phosphorus ingested were due primarily to differences in the amounts of linseed meal in the rations offered, but are affected also by the fact that the low-protein lot did not ingest as much of the concentrates as the others, as is shown from the data of Table II.

The lot variations in the amount of acid-insoluble phosphorus ingested were greater than in that of the total phosphorus. The amount of this form of phosphorus ingested by the medium-protein lot was 192 per cent, and by the high-protein lot 258 per cent of the amount ingested by the low-protein lot. This large variation between the low- and medium-protein lots was due mainly to a difference in the amount of concentrates consumed and to the richness of the concentrates in this form of phosphorus, owing to its higher content of linseed meal. The main cause for the difference between the amounts of acid-insoluble phosphorus ingested by the medium- and high-protein lots was the fact that the acid-insoluble phosphorus content of the concentrates differed considerably, the difference between the quantities of hay or concentrates consumed by the two lots amounting to little.

The amount of acid-soluble phosphorus ingested by the medium-protein lot was 126 per cent and that by the high-protein lot was 132 per cent of that of the low-protein lot. Since the acid-soluble phosphorus content of the concentrates did not differ much, being for the low-, medium-, and high-protein lots 0.141, 0.160, and 0.183 per cent, respectively, the differences between the lots were due largely to the variation in the weights of hay and concentrates consumed.

The amount of inorganic acid-soluble phosphorus ingested by the medium-protein lot was 126 per cent and the amount ingested by the high-protein lot was 139 per cent of the amount ingested by the low-protein lot. The causes of this variation were the same as those for the total phosphorus.

The lot average of the organic acid-soluble phosphorus for the medium-protein lot was 127 per cent and that for the high-protein lot was 119 per cent of the lot average for the low-protein lot. The cause for the difference between the low- and medium-protein lots is traceable to both the amount of the feeds ingested and their richness in this form of phosphorus. The medium-protein lot exceeded the low-protein lot in the consumption of both hay and concentrates, but mainly concentrates; and the hay contained only 0.009 per cent of this form of phosphorus, while the concentrates fed to both the low-protein and the medium-protein lots contained 0.105 per cent. The difference between the organic acid-soluble phosphorus for the medium- and the high-protein lots was due chiefly to a difference in the amount of concentrates consumed. This is clear when it is noted that the average consumption for the medium-protein lot was 693.8 gm. of alfalfa hay and 789.3 gm. of concentrates, while that for the high-protein lot was 697.3 gm. of hay, and 741.4 gm. of concentrates, the medium-protein lot exceeding the high-protein lot in the consumption of concentrates. The organic acid-soluble phosphorus content of the concentrates fed the two lots also had a small influence.

The percentage distribution of the ingested phosphorus among the different kinds is recorded in Table X. It will be noted that the relative amounts of the four different forms of phosphorus ingested by the three lots of lambs varied decidedly.

TABLE X.—*Relative amounts of the different forms of phosphorus ingested by lambs*

[Results expressed in percentage of the total phosphorus ingested]

Ration.	Lamb No.	Total phosphorus.	Acid-insoluble phosphorus.	Acid-soluble phosphorus.		
				Total.	Inorganic.	Organic.
Low-protein...............	468	100	42. 2	57. 8	33. 7	24. 1
	463	100	39. 2	60. 8	40. 1	20. 7
Average................	....	100	40. 7	59. 3	36. 9	22. 4
Medium-protein.............	462	100	51. 1	48. 9	29. 9	19. 0
	458	100	50. 5	49. 5	31. 5	18. 0
Average................	....	100	50. 8	49. 2	30. 7	18. 5
High-protein...............	453	100	57. 2	42. 8	28. 4	14. 4
	451	100	57. 2	42. 8	28. 3	14. 5
Average................	....	100	57. 2	42. 8	28. 3	14. 5

RELATIONS BETWEEN THE PERCENTAGE OF PHOSPHORUS AND OF PROTEIN IN THE FEEDS

Attention has been called to the fact that, while the rations were primarily planned to furnish marked differences in the protein received by the different groups of animals, such differences in the protein were necessarily accompanied by corresponding differences in the amounts and kinds of phosphorus received. The percentages of protein and phosphorus and the ratios of phosphorus to protein in the several feeds offered and in the concentrates of the different lots are given in Table XI.

TABLE XI.—*Phosphorus and protein content of the feeds compared*

Feed.	Phosphorus.	Protein.	Ratio of phosphorus to protein.
	Per cent.	*Per cent.*	
Alfalfa hay......................................	0. 216	12. 50	1 : 57. 9
Corn...	. 269	6. 89	1 : 25. 6
Linseed meal....................................	. 881	31. 79	1 : 36. 1
Concentrates of Lot I...........................	. 300	8. 14	1 : 27. 1
Concentrates of Lot II..........................	. 422	13. 12	1 : 31. 1
Concentrates of Lot III.........................	. 575	19. 34	1 : 33. 6

It is evident from the data given in Table XI that there are marked differences between the ratios of phosphorus to protein in alfalfa hay, corn, and linseed meal. On the other hand, the ratios between the phosphorus and protein in the concentrates fed Lots I, II, and III are not markedly dissimilar.

PHOSPHORUS IN THE FECES AND URINE

PHOSPHORUS CONTENT OF THE FECES

The percentages of the different forms of phosphorus found in the feces are given in Table XII. The average daily excretion of the different forms of phosphorus in the feces and of the total phosphorus in the urines is given in Table XIII.

TABLE XII.—*Different forms of phosphorus in the feces*

[Results expressed in percentage of the fresh substance]

Ration.	Lamb No.	Total phosphorus.	Acid-insoluble phosphorus.	Acid-soluble phosphorus.		
				Total.	Inorganic.	Organic.
Low-protein................	468	0. 400	0. 065	0. 335	0. 321	0. 014
	463	.333	.049	.284	.263	.021
Average................	....	.367	.057	.310	.292	.018
Medium-protein............	462	.416	.042	.374	.338	.036
	458	.342	.065	.277	.254	.023
Average................	....	.379	.053	.326	.296	.030
High-protein................	453	.520	.109	.411	.390	.021
	451	.521	.078	.443	.402	.041
Average................	....	.520	.093	.427	.396	.031

TABLE XIII.—*Different forms of phosphorus excreted by lambs*

[Results expressed in grams per day]

Ration.	Lamb No.	Feces.					Urine: Total phosphorus.
		Total phosphorus.	Acid-insoluble phosphorus.	Acid-soluble phosphorus.			
				Total.	Inorganic.	Organic.	
Low-protein.........	468	2. 56	0. 42	2. 14	2. 05	0. 09	0. 016
	463	3. 24	.48	2. 76	2. 56	.20	.015
Average.......	....	2. 90	.45	2. 45	2. 30	.15	.016
Medium-protein......	462	3. 66	.37	3. 29	2. 97	.32	.012
	458	4. 52	.86	3. 66	3. 36	.30	.018
Average.......	....	4. 09	.61	3. 48	3. 17	.31	.015
High-protein.........	453	5. 11	1. 07	4. 04	3. 83	.21	.018
	451	5. 32	.80	4. 52	4. 10	.42	.014
Average.......	....	5. 22	.94	4. 28	3. 97	.31	.016

As is usually the case with herbivora, by far the largest part of the phosphorus was excreted in the feces. With these lambs the quantities of phosphorus in the urine were all less than 0.02 gm. per day, while those in the feces ranged from 2.6 to 5.3 gm. per day. Evidently, therefore, the phosphorus of the feces is not unassimilated, but in large part is assimilated material which has been excreted into the intestine. Probably the data of the acid-insoluble form more nearly represent those of the undigested phosphorus than do any other figures.

The amounts of total phosphorus excreted and of fecal phosphorus increased with the quantities of phosphorus and protein fed, but the amounts in the urine remained practically constant.

The data for the different forms of phosphorus in the feces, the total phosphorus in the urine, and the total phosphorus stored in the body, expressed in percentage of the total phosphorus ingested, are given in Table XIV.

TABLE XIV.—*Different forms of phosphorus in the feces of lambs, the total phosphorus in their urine, and the total phosphorus stored*

[Results expressed in percentage of total phosphorus ingested]

Ration.	Lamb No.	Total phos- phorus.	Acid- insolu- ble phos- phorus.	Acid-soluble phosphorus.			Total phos- phorus.	Stored phos- phorus.
				Total.	Inor- ganic.	Or- ganic.		
Low-protein...............	{ 468	87. 1	14. 3	72. 8	69. 7	3. 1	0. 5	12. 4
	463	97. 0	14. 4	82. 6	76. 6	6. 0	. 4	2. 5
Average................	......	92. 1	14. 4	77. 7	73. 2	4. 5	. 4	7. 5
Medium-protein.............	{ 462	81. 7	8. 3	73. 4	66. 3	7. 1	. 3	18. 0
	458	88. 5	16. 8	71. 7	65. 8	5. 9	. 4	11. 1
Average................	......	85. 1	12. 5	72. 6	66. 1	6. 5	. 3	14. 6
High-protein...............	{ 453	90. 0	18. 9	71. 1	67. 4	3. 7	. 3	9. 7
	451	91. 9	13. 8	78. 1	70. 8	7. 3	. 2	7. 9
Average................	......	91. 0	16. 4	74. 6	69. 1	5. 5	. 2	8. 8

It is evident from the data given in Table XIV that, on an average, 89.4 per cent of the total phosphorus ingested by the lambs was excreted in the feces. On an average, 75 per cent of the ingested phosphorus of the feeds was excreted in the feces in a form soluble in 0.2 per cent hydrochloric-acid solution, while 69.5 per cent of the total phosphorus consumed was excreted in the feces in the inorganic form.

On account of the small number of animals in each group and the marked individual differences in the data for the different forms of phosphorus in the feces, the total phosphorus in the urine, and the total phosphorus stored in the body expressed in percentage of the total phosphorus ingested, it is impossible to make out significant group differences due to the differences in the quantities of protein and phosphorus ingested.

The data for the percentage distribution of the different forms of phosphorus in the feces in percentage of the total phosphorus are given in Table XV.

TABLE XV.—*Distribution of the forms of phosphorus in the feces of lambs*

[Results expressed in percentage of the total phosphorus]

Ration.	Lamb No.	Total phosphorus.	Acid-insoluble phosphorus.	Acid-soluble phosphorus.		
				Total.	Inorganic.	Organic.
Low-protein...............	468	100. 0	16. 4	83. 6	80. 1	3. 5
	463	100. 0	14. 8	85. 2	79. 0	6. 2
Average...............		100. 0	15. 6	84. 4	79. 6	4. 8
Medium-protein..............	462	100. 0	10. 1	89. 9	81. 1	8. 8
	458	100. 0	19. 0	81. 0	74. 3	6. 7
Average...............		100. 0	14. 5	85. 5	77. 7	7. 8
High-protein...............	453	100. 0	20. 9	79. 1	75. 0	4. 1
	451	100. 0	15. 0	85. 0	77. 1	7. 9
Average...............		100. 0	17. 9	82. 1	76. 1	6. 0

It is apparent that there are no significant group differences in the distribution of the different forms of phosphorus in the feces. The variations within the lots are as great as between lots. The significant facts shown by these results are the relatively small percentage of acid-insoluble phosphorus and the relatively large percentage of acid-soluble inorganic phosphorus in the feces.

The results for the forms of phosphorus in the feces in percentage of the amounts of the same forms ingested are given in Table XVI.

TABLE XVI.—*The forms of phosphorus in the feces of lambs* [a]

[Results expressed in percentage of the amounts of the same forms ingested]

Ration.	Lamb No.	Total phosphorus.	Acid-insoluble phosphorus.	Acid-soluble phosphorus.		
				Total.	Inorganic.	Organic.
Low-protein...............	468	87. 1	33. 9	125. 9	207. 1	12. 7
	463	97. 0	36. 6	136. 0	191. 0	29. 0
Average...............		92. 1	35. 3	131. 0	199. 1	20. 9
Medium-protein..............	462	81. 7	16. 2	149. 3	221. 5	37. 7
	458	88. 5	33. 3	144. 7	208. 7	32. 6
Average...............		85. 1	24. 8	147. 0	215. 1	35. 2
High-protein...............	453	90. 0	32. 9	166. 3	237. 9	25. 6
	451	91. 9	24. 2	182. 3	250. 0	50. 0
Average...............		91. 0	28. 6	174. 3	244. 0	37. 8

[a] Values above 100 indicate larger amounts excreted in the given form than were ingested in that form.

The variations for the forms of phosphorus in the feces in percentage of the amounts of the same forms ingested are marked, both within the lots and between the lots. The cause of these variations is not apparent from the data available. It is, however, evident that the forms of phosphorus of the feeds underwent profound changes during the processes of digestion and metabolism. A large proportion of the acid-insoluble phosphorus of the feeds was converted into acid-soluble phosphorus and a large part of the soluble organic phosphorus was also changed into acid-soluble inorganic phosphorus.

PHOSPHORUS BALANCE

The daily phosphorus balances of the lambs for the continuous 12-day metabolism test are given in Table XVII.

TABLE XVII.—*Daily phosphorus balances (in grams) of lambs in metabolism test*

Ration.	Lamb. No.	Intake.	Output.			Balance.
			Feces.	Urine.	Total.	
Low-protein...............	{ 468	2. 94	2. 56	0. 016	2. 576	0. 364
	{ 463	3. 34	3. 24	. 015	3. 255	. 085
Average...............	....	3. 14	2. 90	. 016	2. 916	. 224
Medium-protein...........	{ 462	4. 48	3. 66	. 012	3. 672	. 808
	{ 458	5. 11	4. 52	. 018	4. 538	. 572
Average...............	....	4. 80	4. 09	. 015	4. 105	. 695
High-protein..............	{ 453	5. 68	5. 11	. 018	5. 128	. 552
	{ 451	5. 79	5. 32	. 014	5. 334	. 456
Average...............	....	5. 74	5. 22	. 016	5. 236	. 504

All of the lambs showed positive phosphorus balances. Even the low-protein lot, which, on an average, ingested 3.14 gm. of phosphorus per day, showed a balance of 0.224 gm. per day. These low-protein lambs were fed the same feeds in the same quantities per 100 pounds of live weight from weaning time, June 25, to January 28, a period of 217 days, as they were fed during this metabolism test. During the main feeding experiment they made an average daily gain of 0.28 pound per head per day. From these satisfactory gains for a period of 7 months and the positive phosphorus balance shown during the metabolism period of 12 days, it is probable that the phosphorus requirement for the normal growth and fattening of lambs is not more than 3 gm. per day per 100 pounds of live weight.

From the available data it is not apparent that there was any correlation between the quantities of phosphorus stored and the quantities of protein and phosphorus ingested.

SUMMARY

(1) There are marked differences in the percentages of the different forms of phosphorus occurring in alfalfa hay, corn, and linseed meal, and in the ratio of phosphorus to protein in these feeds. A large part of the phosphorus of alfalfa hay consists of the acid-soluble inorganic form; the phosphorus of corn is equally divided between acid-insoluble and acid-soluble, the soluble being largely organic; and the phosphorus of linseed meal is largely in the acid-insoluble form, the soluble being about equally divided between inorganic and organic phosphorus.

(2) Upon a ration of alfalfa hay, corn, and linseed meal lambs excrete in the urine only two-tenths to five-tenths of 1 per cent of the total phosphorus ingested.

(3) The forms of phosphorus excreted in the feces of lambs show that the forms of phosphorus in the feeds consumed undergo marked qualitative and quantitative changes during the processes of digestion and metabolism. A large proportion of the acid-insoluble phosphorus of the feeds is converted into acid-soluble phosphorus, and a large part of the soluble organic phosphorus is also changed into acid-soluble inorganic phosphorus. Therefore, there is relatively only a small percentage of acid-insoluble phosphorus and a relatively large percentage of inorganic acid-soluble phosphorus in the feces.

(4) The results of this metabolism experiment, together with those of the main feeding experiment of 217 days' duration, indicate that the phosphorus requirement for the normal growth and fattening of lambs does not exceed 3 gm. per day per 100 pounds of live weight.

(5) There is no evidence of correlation between the amounts of phosphorus retained in the body, on the one hand, and the amounts of phosphorus ingested, the amounts of protein ingested, or the body weights of lambs, on the other hand.

(6) Variations in the quantity of digestible protein consumed from 1.56 to 3.19 pounds per 1,000 pounds of live weight per day by lambs do not influence significantly the forms of phosphorus in the feces, the total phosphorus in the urine, or the total phosphorus stored in the animal body, expressed in percentage of the total phosphorus ingested.

A BACTERIAL DISEASE OF LETTUCE

[A PRELIMINARY REPORT]

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In January, 1915, some diseased lettuce plants (*Lactuca sativa*) were sent to the United States Department of Agriculture from Nairn, La. The letter accompanying them stated that the disease was ruining the lettuce crop in that section, that about 200 acres of lettuce plants were badly infected, and that the fields looked as though a fire had swept through them.

At first the growers thought the disease was due to the excessive use of cottonseed meal, but it was reported in fields where no cottonseed meal was used. It occurred on high land, but was most prevalent on flat land. There had been excessive rainfall for three months in the affected region; however, there were fields within 10 feet of the infected area that showed no visible trace of infection.

The plants received by the Department were full-grown heads with some of the outer leaves entirely shriveled and dried and some of them in a soft-rotted condition. The centers of the heads were sound, but between the center and these dead outer leaves were others affected in varying degrees. In some places there were numerous separated spots with a water-soaked appearance. In other places the spots had fused. Portions of many leaves were in a bad condition, while other parts of the same leaves were sound.

Razor sections of areas showing the earliest evidence of the disease were examined under the microscope, and numerous bacteria were found in the cells and between them. Fungus threads were not detected. In the advanced stages of the disease the palisade cells and the loose parenchyma cells had collapsed. Some of the younger diseased areas were used for isolating the organism presumed to cause the trouble, the isolation being made by means of agar-poured plates. The organism so obtained was proved to be infectious.

Colonies appeared three days after pouring the plates. Those colonies which produced the disease when they were inoculated into healthy lettuce plants were later studied carefully on agar plates. When very young they are round with entire margins smooth, translucent, cream-white in reflected light, bluish in transmitted light, with fish-scale-like markings which are not always present and which do not seem to be on the surface. These markings disappear as the colonies get older. When 3 days old,

many colonies have a denser margin which is lighter colored than the center. When older, the center is not always uniform in color. It may have yellowish bands or mottlings and patches of the lighter margin color in it. There is not always a definite light margin, and some colonies seem quite uniform throughout. The colonies range from 3 to 5 mm. in diameter. On agar stroke this mottling is present when the culture is young, but disappears in both stroke and plate colony as they get older. Inoculations have been made with the mottled colonies and those uniformly colored—that is, either cream-colored or bluish throughout. All types are infectious.

Using subcultures from single colonies, the disease was reproduced with this organism by needle-prick inoculations four different times (12 plants) and twice by spraying water suspensions of it on middle-aged plants growing in the greenhouses (7 plants). Checks held under the same condition of heat and moisture remained healthy. Reisolation inoculations by means of needle pricks were also made, and these, too, were successful.

The organism is a bacterium,[1] motile by means of from one to three polar flagella; it is non-gas-forming in peptone water with the sugars and alcohols tried (dextrose, lactose, saccharose, maltose, mannit, and glycerin). It did not cloud the closed end in any of the fermentation tubes, but it clouds beef bouillon $+15$ in less than 24 hours at $23°$ C. when transfers are made from beef bouillon. In 10 days the bouillon has become a lime-green color.[2] The organism clears sterile milk in 15 days without coagulation, the cleared fluid becoming a pale turtle-green color. It blues litmus milk and will grow in peptone-beef bouillon at temperatures ranging from $1.5°$ to $34.5°$ C., though it will not grow in bouillon at $36°$ C. The thermal death point lies between $48°$ and $49°$ C. It grows well in Uschinsky's and Fermi's solutions, changing them to pale Veronese green and water green in 3 to 5 days, but grows very faintly in Cohn's solution. The organism liquefies gelatin slowly at $18.5°$ C., one-half of the gelatin in test-tube cultures being liquefied in 10 days. On potato cylinders it produces a fleeting dark blue-green color. This striking color reaction develops promptly and disappears on the sixth day or earlier. It grows in bouillon over chloroform, tolerates malic, tartaric, and citric acid (0.1 to 0.2 per cent) added to neutral beef bouillon, but will not grow in neutral beef bouillon containing 0.3 per cent of these acids. It grows readily in neutral and in beef bouillon $+5$, moderately in -10 and -18, faintly in -20, but will not grow in -22 beef bouillon.

The organism withstands a limited amount of drying. A drop of a 1-day-old bouillon culture smeared over sterile cover glasses and kept in

[1] This use of the genus Bacterium is in accordance with the system of classification proposed by Erwin F. Smith *in his* Bacteria in Relation to Plant Diseases. v. 1, p. 171. Washington, D. C., 1905. (Carnegie Inst. Wash. Pub. 27.)

[2] Ridgway, Robert. Color Standards and Color Nomenclature. 43 p., 53 col. pl. Washington, D. C., 1912.

the dark at room temperature (20° to 23° C.) will produce growth up to the eleventh day of drying when such covers are placed in tubes of beef bouillon.

It is not especially sensitive to sunlight. Petri dishes, one half covered with black paper and exposed bottom up to the noon-day sun in April on a sack of ice, developed 15 to 30 colonies on the uncovered parts exposed for 30 minutes, but none at 40 minutes. The covered part of the 30-minute plates developed from 130 to 150 colonies; that of the 40-minute plates developed from 30 to 55 colonies.

The organism likewise grows in neutral beef bouillon containing 3 and 4.5 per cent of sodium chlorid, but does not grow in the same medium with 5 per cent of common salt. It produces indol, but less abundantly than *Bacillus coli*, and does not reduce nitrates.

Stained from young agar cultures, the organism is a short rod with rounded ends. It is less than 1 to 1.25μ in diameter and 1.25 to 3μ long. It occurs singly, in pairs, and also in chains. Spores have not been observed. The organism stains readily with carbol fuchsin, gentian violet, methyl violet, and methylene blue. It is Gram-positive, and is not acid-fast. The flagella were stained by Loeffler's flagella stain.

A bacterial disease of lettuce has been reported from the Vermont, the Massachusetts, the Florida, and the North Carolina experiment stations. Pietro Voglino (1904)[1] in Italy has reported a bacterial disease of lettuce and named his organism "*Bacillus lactuacae*." As the description of the organism reported in his paper does not agree with our own (pink, nonliquefying, spore-bearing, etc.), it is clear that the Louisiana organism is not the same as the Italian, but is possibly the same as some one of the unnamed forms previously isolated in this country and not carefully described. The name "*Bacterium viridilividum*, n. sp.," is suggested for the one under consideration, owing to its peculiar appearance on steamed potato.

For purposes of orientátion, a short account of the literature on bacterial diseases of lettuce follows:

L. R. Jones (1893)[1] has given an account of a bacterial stem-rot of lettuce. A large bacillus was found in the diseased stems, but was not isolated. He reproduced the disease (1) by planting healthy plants in soil inoculated with fragments of lettuce plants affected by "stem rot," (2) by crushing a diseased lettuce head in a little water and pouring this water about the roots of healthy plants.

G. E. Stone (1907) mentions a bacterial disease of lettuce leaves which had been investigated by Mr. Percival C. Brooks six years earlier. It is stated that Mr. Brooks isolated an organism and produced positive results from inoculation experiments. As the disease was believed to

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[1] Bibliographic citations in parentheses refer to "Literature cited," p. 478.

be of little consequence, no extensive study was made. There is no description of his organism.

F. L. Stevens (1908), in a short report on a bacterial disease of lettuce, states that the bacteria isolated were rather long rod forms. His attempts at inoculation were unsuccessful.

H. S. Fawcett (1908) also reports a bacterial disease of lettuce. He likewise isolated an organism and reproduced the disease. His colonies on standard peptonized agar had indefinite margins and pearl-white foci. The organism stained readily in carbol fuchsin and aqueous gentian violet, but with difficulty in methylene blue.

O. F. Burger (1912) describes a bacterial disease of lettuce which he says is caused by a species of Pseudomonas. The disease begins at the center of the head, which blackens and then becomes soft. In the seed bed the disease appears as small black spots on the leaves. This does not seem to be the type of disease that occurred in Louisiana this year. Burger states that cultures of the bacteria were made and healthy lettuce plants were inoculated. In 10 days the inoculated plants were black and pulpy, while the checks were still healthy.

Bacterium viridilividum does not agree with the descriptions of any of the organisms mentioned by these writers. Voglino's (1904) organism evidently does not liquefy gelatin, and the colonies in lettuce gelatin change from an ivory-white color to a rosy tint. *B. viridilividum* liquefies gelatin, and is never ivory white or of a rosy tint. Fawcett's (1908) organism produces colonies with indefinite margins and pearl-white foci and stains with difficulty in methylene blue. *B. viridilividum* has definite margins, no pearl-white foci, and stains readily in methylene blue. Stevens's (1908) organism is a long rod form; *B. viridilividum* is a short rod. Stevens's inoculations were not successful. Jones (1893), Brooks (Stone, 1907), and Burger (1912) did not describe their organisms.

LITERATURE CITED

BURGER, O. F.
   1912. Lettuce rot. Fla. Agr. Exp. Sta. Press Bul. 200, 2 p.
FAWCETT, H. S.
   1908. Lettuce disease. *In* Fla. Agr. Exp. Sta. Rpt., [1907]/08, p. lxxx–lxxxvii, pl. 4–5.
JONES, L. R.
   1893. A bacterial "stem-rot" of lettuce. *In* Vt. Agr. Exp. Sta. 6th Ann. Rpt., 1892, p. 87–88.
STEVENS, F. L.
   1908. A serious lettuce disease. *In* N. C. Agr. Exp. Sta. 30th Ann. Rpt., [1906]/07, p. 72.
STONE, G. E.
   1907. Bacterial disease of lettuce. *In* Mass. Agr. Exp. Sta. 19th Ann. Rpt., [1906], p. 163–164.
VOGLINO, Pietro.
   1904. Sulla batteriosi delle lattughe. *In* Ann. R. Accad. Agr. Torino, v. 46, 1903, p. 25–33, 4 fig.

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DEGREE OF RESEMBLANCE OF PARENTS AND OFF-SPRING WITH RESPECT TO BIRTH AS TWINS FOR REGISTERED SHROPSHIRE SHEEP

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INTRODUCTION

In 1906 Rommel and Phillips [1] reported an investigation into the inheritance of the size of litter in the female line in Poland China sows based on data of the American Poland China Record. In 1907 there was published a statistical analysis of data from the records of Weldon [2] on heredity in the size of litters in mice.

The present investigation resembles somewhat the above investigations in that it is concerned with the question of the likeness of animals and their offspring with respect to being born as singles, twins, or triplets. We are concerned with the question whether and to what extent the offspring of parents born in twins and of grandparents born in twins are more likely to be twins than if these ancestors are born as singles.

Stated in another way, when we know that certain animals of a given breed or class and certain of their ancestors are born as twins or triplets, is there greater probability that the offspring will be twins or triplets than if the animals were born as singles?

Our problem may be made clearer by asking a question that covers only part of the problem: Does the subclass of sheep of a certain breed which consists of those born as twins tend to beget twins in larger proportion than the subclass that consists of those born as singles?

In the Journal of the Royal Agricultural Society of England [3] there appears a statement of some of the possible causes that are favorable to the production of twins. It is there stated that "there is some reason to believe that twin lambs produce more twins than single lambs, and that the influence of heredity is brought to bear." The main purpose

[1] Rommel, G. M., and Phillips, E. F. Inheritance in the female line of size of litter in Poland China sows. *In* Biometrika, v. 5, pt. 1/2, p. 203-205. 1906.

[2] [Weldon, W. F. R.] On heredity in mice from the records of the late W. F. R. Weldon. *In* Biometrika, v. 5, pt. 4, p. 436-449. 1907.

[3] Heape, Walter. Abortion, barrenness, and fertility in sheep; an abstract of records obtained for the year 1896-97. *In* Jour. Roy. Agr. Soc. England, s. 3, v. 10, pt. 2, p. 236. 1899.

of the present paper is to submit an analysis of data with a view to testing the foundations of such a belief when we are dealing with a class of pure-bred sheep.

To indicate birth as a single, in twins, or in triplets, we use the symbols "1" for single, "2" for twin, and "3" for triplet.

SOURCE OF DATA

The source of all our data is the American Shropshire Sheep Record. We have taken individuals with numbers from 325502 to 344869,[1] and have looked up their parents and grandparents with respect to the state of birth in singles, twins, and triplets.

All cases are omitted where either parent is imported, for the reason that the English records do not show whether an animal is born single, in twins, or in triplets.

For each of the offspring above mentioned, with American-born parents, we have made a card, showing whether this animal is born in our symbolism as a 1, 2, or 3 and showing in which of the states 1, 2, or 3 its parents and nonimported grandparents are born.

DISCRIMINATION IN FAVOR OF OR AGAINST RECORDING TWINS

In beginning this investigation we made some inquiry concerning possible discrimination in favor of or against the recording of twins, and found no reason to believe that there existed such discrimination. In the Journal of the Royal Agricultural Society of England,[2] we find that 46.84 per cent of Shropshire ewes involved in the data there analyzed have twins. This would mean that nearly 64 per cent of the lambs born are twins. But the percentage of lambs born as twins and triplets that we have found in fairly large classes of offspring does not seem to exceed 43, which is very different from 64 per cent. The difference seems to mean that either Shropshires in America are less fertile than in England or there is discrimination against twins in the matter of recording. This does not mean that the discrimination is made directly against twins, but probably in an indirect manner for some such reason as the better development of singles when the selections are made. However, for the main purpose of our problem, we are concerned with the elimination of discriminations where one would record twins or singles because certain ancestors are twins or singles—that is, we are concerned with the elimination of the kind of discrimination that would give an affirmative answer to the question, Do breeders tend to record an increased or decreased proportion of twins on account of the fact that certain parts of the ancestry consist of twins or of singles? Such discrimination is doubtless much less likely than a more general sort of discrimination that would lead to the recording of a larger or smaller proportion of twins than we find in a random sample.

[1] American Shropshire Sheep Record, v. 25, p. 1–1314. 1912. [2] Heape, Walter. Op. cit., p. 235.

There are some cases where owners of sires of large production have recorded from the same sire in two consecutive years all singles in one of the years and nearly all twins in the other year. Such cases make it appear that these few owners tend to select twins or singles in making records. The following section, "Repetition of sires and paternal grandparents," shows how we have treated such special cases.

REPETITION OF SIRES AND PATERNAL GRANDPARENTS

One sire may belong to a large number of recorded offspring, although this happens in relatively few cases; for example, there is a case in which one sire belongs to as many as 135 recorded offspring in the period for which we have examined offspring, and this fact is not to be neglected in making a critical examination of our data. In fact, a few such cases of large production with discrimination against either twins or singles might vitiate our results on the correlation of offspring with sires and grandsires. On account of the possibility of error from this source, we arrange a table, separating the offspring of each sire into singles and twins. From this arrangement of data it is fairly clear that certain cases of extreme percentages of singles or twins from a sire of large production should be excluded from data used in the calculations of statistical constants. We fix criteria somewhat arbitrarily as follows:

(1) Cases are excluded where a sire has more than 10 recorded offspring that are all singles or all twins.

(2) Cases are excluded where a sire has more than 20 recorded offspring if the *difference* in percentage of twins among this offspring and among the general population of offspring is more than three times the probable error of the difference.

While we exclude from our calculations the part of the data just mentioned, we give such data in Tables XV, XVI, XVII, XXXV, and XXXVI, in order that anyone who may consider the above criteria for exclusion too stringent or too lenient may have available for criticism such excluded data. Tables I, IX to XIV, XVIII, and XXVI to XXXII involve data about sires and do not include data concerning those cases of high-producing sires which are to be excluded from our calculations of statistical constants.

ANALYSIS OF DATA FOR SIRES, DAMS, AND OFFSPRING

Table I(A) shows the frequencies with which sires and dams born in states 1–1, 1–2, 2–1, 2–2, 1–3, 3–1, 2–3, 3–2, or 3–3 beget recorded offspring born in states 1, 2, or 3. In this notation the first number of a pair refers to the sire and the second to the dam. Thus, 1–2 means that the sire is born as a single and the dam as a twin. To illustrate further the meaning of the table, consider the number 1,276 in the column marked "1" and in the row marked "2–1." This means that 1,276 offspring out of the total of 9,291 are singles with twin sires and single dams.

TABLE I(A).—*Correlation between size of litter in which offspring are born and size of litter in which pairs of parents are born*

Sires and dams.	Offspring.			Total.
	1	2	3	
1–1	2,018	1,026	15	3,059
1–2	1,430	996	10	2,436
2–1	1,276	800	12	2,088
2–2	867	661	22	1,550
1–3	24	29	....	53
3–1	3	14	....	17
2–3	21	20	3	44
3–2	10	21	2	33
3–3	9	2	....	11
Total	5,658	3,569	64	9,291

$$r = 0.0880 \pm 0.0070,$$

where r is the correlation coefficient between the sum of numbers in litters in which sire and dam are born and the number in corresponding litters in which offspring are born.

Means of arrays of offspring:

(1) When sire and dam are singles.................... 1.3452 ± 0.0059.
(2) When sire is single and dam is twin.............. 1.4171 ± 0.0067.
(3) When sire is twin and dam is single.............. 1.3946 ± 0.0073.
(4) When sire is twin and dam is twin............... 1.4548 ± 0.0088.
(5) When either sire or dam is a triplet............... 1.6076 ± 0.030.
Mean of all offspring......................... 1.3979 ± 0.0035.

Table I(B) simply exhibits percentages of singles, twins, and triplets among offspring that belong to different kinds of parents. It seems from this table that the percentage of twins among offspring of twin parents is greater than that among the offspring of single parents. From the means of arrays of offspring in Table I(A), we note that the differences are significant when judged by their probable errors. The correlation between the sum of the numbers of lambs born in the litters in which the two parents are born and the number in the litter in which the offspring is born, given by

$$r = 0.0880 \pm 0.0070,$$

is a significant positive correlation.

TABLE I(B).—*Percentages of offspring born in states 1, 2, and 3, to correspond to states 1–1, 1–2, 2–1, . . . of sire and dam*

Sires and dams.	Offspring.		
	1	2	3
1–1	65.97	33.54	0.50
1–2	58.70	40.89	.41
2–1	61.11	38.31	.58
2–2	55.93	42.65	1.42
(a)	42.40	54.43	3.17
Based on total	60.90	38.41	.69

[a] This row of percentages is obtained by grouping together all offspring where either parent is a triplet. Even when thus grouped, the number of offspring is small.

TABLES FOR DAMS AND OFFSPRING

Table II(A) shows the frequencies with which recorded offspring born in states 1, 2, and 3 have dams born in these states, or the frequencies with which dams born in states 1, 2, or 3 beget recorded offspring in states 1, 2, or 3.

TABLE II(A).—*Correlation between size of litter in which offspring are born and size of litter in which dams are born*

Dams.	Offspring.			Total.
	1	2	3	
1....................................	3,784	2,046	27	5,857
2....................................	2,586	1,930	40	4,556
3....................................	58	58	3	119
Total.......................	6,428	4,034	.	70	10,532

$r = 0.0869 \pm 0.0065$.

Means of arrays of offspring:
    (1) When dams are singles........................... 1.3585 ± 0.0043.
    (2) When dams are twins............................ 1.4412 ± 0.0051.
    (3) When dams are triplets.......................... 1.538 ± 0.034.

Table II(B) simply converts into the form of percentages the frequencies given in Table II(A), so that the essential points of interest may be grasped more easily. From means of arrays of offspring in Table II(A), we note that there is a significant tendency for twin dams to produce a larger percentage of twins than is produced by single dams. The correlation is given by

$$r = 0.0869 \pm 0.0065.$$

TABLE II(B).—*Percentages of offspring born in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the dams*

Dams.	Offspring.		
	1	2	3
1..	64.61	34.93	0.46
2..	56.76	42.36	.88
3..	48.7	48.7	2.5

ANALYSIS OF DATA FOR DAMS, MATERNAL GRANDPARENTS, AND OFFSPRING

Table III(A) represents the distribution of offspring, dams, and maternal granddams with respect to states 1, 2, and 3. To illustrate the meaning of the table, consider the number 1,393 in the column headed 1 and in the row marked 1–2. This means that there are 1,393 of the

total number of offspring that are singles with single dams and twin maternal granddams.

Dams and granddams are repeated so as to let all the recorded offspring appear. Since we do not know in which of the states 1, 2, or 3 imported sheep were born, we have to omit all cases of imported granddams.

TABLE III(A).—*Correlation between offspring and maternal granddams* [a]

Dams and maternal granddams.	Offspring.			Total.
	1	2	3	
1–1	2,170	1,098	17	3,285
1–2	1,393	796	7	2,196
2–1	1,312	935	12	2,259
2–2	1,126	881	22	2,029
1–3	34	30	....	64
3–1	19	20	1	40
2–3	27	29	4	60
3–2	26	21	2	49
3–3	3	8	....	11
Total	6,110	3,818	65	9,993

[a] This abbreviated heading will be used for tables that follow. The correlation refers to that between sizes of litters in which the classes are born.

Means of arrays of offspring:

    (1) When dams and granddams are singles............ 1.3446±0.0057.
    (2) When the dams are singles and granddams twins.... 1.3689±0.0070.
    (3) When the dams are twins and granddams are singles. 1.4245±0.0071.
    (4) When the dams are twins and granddams are twins.. 1.4559±0.0078.
    (5) When either dam or granddam is a triplet.......... 1.545 ±0.037.

Table III(B) exhibits the percentages of offspring born in states 1, 2, and 3 for dam and maternal granddams born in various states.

TABLE III(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1–1, 1–2, 2–1, 2–2, of dams and maternal granddams*

Dams and maternal granddams.	Offspring.		
	1	2	3
1–1	66.06	33.42	0.52
1–2	63.43	36.25	.32
2–1	58.08	41.39	.53
2–2	55.50	43.42	1.08
(a)	48.66	48.21	3.13

a See footnote, Table I(B).

It is to be noted from Table III(B) that the percentage of twins varies from 33.42 to 48.21, there being a gradual increase in percentage of twins when twins and triplets occur as dams and granddams. The greatest influence we should ascribe to the twin dam, as the occurrence of the twin granddam does not increase the percentage nearly as much as does that of the twin dam.

We hesitate to calculate a correlation coefficient from Table III(A), for the reason that we have no single number to mark each selected array and would not feel justified in giving an interpretation to a correlation coefficient obtained by taking the sum or mean value of the two numbers associated with each array. We might weight the dam and granddam, but such a system of weights would in the present state of knowledge be little better than a set of guesses.

We may note from the means of arrays and their probable errors that there can be no reasonable doubt about the significance of the difference in means when in one case the dams are singles and in the other they are twins. Further, it is to be observed that there appears to be a slight influence of twin maternal granddams in increasing mean values. Such influence is not, however, nearly so surely established as is the influence of the dams. In fact, the difference in the case of single and twin granddams with single dams is not quite three times the probable error.

Table IV shows the frequencies with which maternal granddams born in states 1, 2, or 3 beget recorded offspring born in states, 1, 2, or 3. There is a significant correlation given by

$$r = 0.0433 \pm 0.0067.$$

TABLE IV.—*Correlation between offspring and maternal granddams*

Maternal granddams.	Offspring.			Total.
	1	2	3	
1	3,501	2,053	30	5,584
2	2,545	1,698	31	4,274
3	64	67	4	135
Total	6,110	3,818	65	9,993

Means of arrays of offspring:
 (1) When the maternal granddams are singles.......... 1.3784±0.0045.
 (2) When the maternal granddams are twins............ 1.4120±0.0052.
 (3) When the maternal granddams are triplets.......... 1.556 ±0.033.

Table V(A) represents the distribution of offspring, dams, and maternal grandsires with respect to states 1, 2, and 3 in a manner analogous to that of Table III(A). Here again, when dams are twins, we note

from the percentages in Table V(B) that the percentage of twins increases, but there is no significant difference between the percentages of twins produced by single dams whose sires are singles and by single dams whose sires are twins. Further, there appears to be no significant difference between the percentages of twins produced by twin dams whose sires are singles and by twin dams whose sires are twins. Further, Table VI shows the frequencies with which maternal grandsires born in states 1, 2, or 3 beget offspring born in states 1, 2, or 3. From this table we obtain the correlation coefficient

$$r = 0.0042 \pm 0.0073,$$

which is so small that we are unable to assert the existence of any significant correlation.

TABLE V(A).—*Correlation between offspring and maternal grandsires*

Dams and maternal grandsires.	Offspring.			Total.
	1	2	3	
1–1	1,751	929	2	2,682
1–2	1,159	596	13	1,768
2–1	1,159	894	17	2,070
2–2	818	596	19	1,433
1–3	38	27	1	66
3–1	20	32	3	55
2–3	25	21	1	47
3–2	21	18	....	39
3–3	....	....	....	....
Total	4,991	3,113	56	8,160

Table V(A) does not include so many records as previous tables because imported grandsires can not be included.

TABLE V(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1–1, 1–2, 2–1, ... of the dams and maternal grandsires*

Dams and maternal grandsires.	Offspring.		
	1	2	3
1–1	65. 29	34. 64	0. 07
1–2	65. 55	33. 71	. 74
2–1	55. 99	43. 19	. 82
2–2	57. 08	41. 59	1. 33
(a)	50. 24	47. 34	2. 42
Based on total	61. 16	38. 15	. 69

a See footnote, Table I (B).

TABLE VI.—*Correlation between offspring and maternal grandsires*

Maternal grandsires.	Offspring.			Total.
	1	2	3	
1	2,930	1,855	22	4,807
2	1,998	1,210	32	3,240
3	63	48	2	113
Total	4,991	3,113	56	8,160

$$r = 0.0042 \pm 0.0073.$$

Means of arrays of offspring:

     (1) When maternal grandsires are singles .... 1.3950±0.0059.
     (2) When maternal grandsires are twins............... 1.3932±0.0049.
     (3) When maternal grandsires are triplets............. 1.460 ±0.034.

Tables VII(A) and VII(B) are correlation tables for maternal grand-dams and dams. These tables show correlation between dams and offspring just as Tables II(A) and II(B) do, the only difference being that the offspring is in this case limited to the females who themselves become dams.

TABLE VII(A).—*Correlation between dams and maternal granddams*

Maternal granddams.	Dams.			Total.
	1	2	3	
1	2,754	1,813	37	4,604
2	1,810	1,570	45	3,425
3	54	45	9	108
Total	4,618	3,428	91	8,137

$$r = 0.0770 \pm 0.0074.$$

TABLE VII(B).—*Percentages of dams in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the maternal granddams*

Maternal granddams.	Dams.		
	1	2	3
1	59.82	39.38	0.80
2	52.85	45.84	1.31
3	50.00	41.67	8.33
Based on total	56.75	42.13	1.12

The correlation coefficient computed from the data of Table VII (A) is

$$r = 0.0770 \pm 0.0074.$$

This correlation is significant when judged by its probable error, and it does not differ significantly from the correlation between dams and offspring as found from Table II(A).

Table VIII(A) is a correlation table for maternal grandsires and dams. This is the case of the correlation of sires with female offspring with respect to birth as singles, twins, or triplets. The correlation coefficient is

$$r = 0.0066 \pm 0.0075.$$

This result makes it somewhat probable that there is no correlation between sires and their female offspring with respect to the character in question. We shall investigate this matter further by the separation of offspring with regard to sex. (See Tables XXV and XXXI.)

TABLE VIII(A).—*Correlation between dams and maternal grandsires*

Maternal grandsires.	Dams.			Total.
	1	2	3	
1	2,255	1,665	45	3,965
2	1,514	1,145	31	2,690
3	57	48	....	105
Total	3,826	2,858	76	6,760

$$r = 0.0066 \pm 0.0075.$$

TABLE VIII(B).—*Percentages of dams in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the maternal grandsires*

Maternal grandsires.	Dams.		
	1	2	3
1	56.87	41.99	1.14
2	56.28	42.57	1.15
3	54.29	45.71	....
Based on total	56.60	42.28	1.12

ANALYSIS OF DATA FOR SIRES AND OFFSPRING

Tables IX(A) and IX(B) are analogous to Tables II(A) and II(B), where the dams are replaced by sires and where certain abnormal cases are excluded in accord with criteria given under "Repetition of sires and paternal grandparents."

TABLE IX(A).—*Correlation between offspring and sires*

Sires.	Offspring.			Total.
	1	2	3	
1	3, 472	2, 051	25	5, 548
2	2, 164	1, 479	37	3, 680
3	22	39	2	63
Total........................	5, 658	3, 569	64	9, 291

$$r = 0.0527 \pm 0.0070.$$

Means of arrays of offspring:
    (1) When the sire is a single........................... 1. 3787 ± 0. 0045.
    (2) When the sire is a twin........................... 1. 4220 ± 0. 0057.
    (3) When the sire is a triplet........................ 1. 683 ± 0. 045.

TABLE IX(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the sires*

Sires.	Offspring.		
	1	2	3
1	62. 58	36. 97	0. 45
2	58. 80	40. 19	1. 01
3	34. 92	61. 90	3. 17
Based on total	60. 90	38. 41	0. 69

While the same sort of tendencies are to be noted in the examination of these tables as with Tables II(A) and II(B), it seems that the tendency of twins to produce twins is less marked and that the correlation is given by

$$r = 0.0527 \pm 0.0070.$$

If we had excluded none of the offspring of large producing sires with an abnormal proportion of recorded twins or singles, we should have obtained

$$r = 0.0294 \pm 0.0066.$$

We think there is, however, little doubt that it would be improper to include for purposes of calculation at least most of the cases we have excluded.

We may well note the increase in means of arrays for twin and triplet sires.

In order to examine a little more critically into the question whether twin sires tend to have a larger number of twin offspring than do single sires, we have invented an index number for each sire. This number is

formed by marking with a "1" each of the offspring born single and with a "2" each one born in twins and next by finding the arithmetical mean of the numbers that belong to the offspring of any sire. Clearly, if all the offspring of a sire were singles, under this scheme his index number would be 1, while if all were born in twins, his index number would be 2.

Next, finding the arithmetical mean of the index numbers for all the sires, we have a sort of measure that enables us to compare the tendency of twins to produce twins and without giving weight to repetitions of the sires to correspond to each individual offspring.

It results that the following numbers are associated with sires born as singles and sires born twins.

For all sires born in singles, we have the mean value 1.3300 ± 0.0078.

For all sires born in twins, we have the mean value 1.380 ± 0.010.

These results show a larger relative production of twins by twin sires than by single sires. The difference, however, is only about four times the probable error of the difference. This surely means that it takes rather large numbers to establish the significance of the difference if such difference exists.

In making the application of this scheme of index numbers, we came upon an interesting form of frequency distribution that seems to occur but rarely. In these distributions the most frequent values are at the index numbers 1 and 2. The modal values are thus at or near the ends of the range. The following are the distributions:

Sires born in singles.		Sires born in twins.	
Index number.	Frequency.	Index number.	Frequency.
1. 0	586	1. 0	342
1. 1	19	1. 1	14
1. 2	57	1. 2	19
1. 3	66	1. 3	56
1. 4	51	1. 4	33
1. 5	72	1. 5	49
1. 6	47	1. 6	25
1. 7	68	1. 7	52
1. 8	30	1. 8	26
1. 9	27	1. 9	11
2. 0	170	2. 0	126
2. 3	1	2. 1	2
2. 5	2	2. 3	1
3. 0	4	2. 4	1
		2. 5	2
		3. 0	8

The large numbers at or near the ends of the range are to be accounted for, in part at least, by the fact that a considerable number of sires have only one recorded offspring. In such cases the index number must be 1 or 2. It can not have an intermediate value. Next, with only a few

offspring recorded, say, with two individual offspring recorded, the index number may fall only into one of the three classes, 1, 2, or 1.5, when we neglect the rare case of triplets. For index numbers approaching 1 but not equaling 1, a larger number of offspring is required; for example, it would require at least 7 offspring, 6 born in singles and 1 in twins, to enter the class 1.1, and it would require at least 6 twins and 1 single to enter class 1.9.

These facts seem to account for the smaller numbers contiguous to the ends of the range than are found at other intermediate points.

ANALYSIS OF DATA ON SIRES AND PATERNAL GRANDPARENTS

Tables X (A) and X (B) represent the distribution of paternal grand-dams and sires of offspring treated in Tables II and IX. The means of arrays show that the tendency to produce twins is increased by the use of twin sires instead of singles sires, but that this tendency is not changed significantly by the use of twin paternal granddams instead of single paternal granddams.

This result is further supported by obtaining from Table XI(A) for the correlation coefficient

$$r = -0.0100 \pm 0.0084.$$

We are thus unable to assert the existence of a significant correlation.

TABLE X(A).—*Correlation between offspring and paternal granddams*

Sires and paternal granddams.	Offspring.			Total.
	1	2	3	
1–1................................	1, 574	937	15	2, 526
1–2................................	1, 269	742	8	2, 019
2–1................................	855	593	26	1, 474
2–2................................	894	578	7	1, 479
1–3................................	43	26	1	70
3–1................................	20	21	2	43
2–3................................	36	32	....	68
3–2................................	2	21	....	23
3–3................................	....	....	....	....
Total.......................	4, 693	2, 950	59	7, 702

Means of arrays of offspring:
   (1) When sire and paternal granddam are singles........ 1. 3828±0. 0067.
   (2) When sire is a single and paternal granddam is a twin. 1. 3754±0. 0072.
   (3) When sire is a twin and paternal granddam is a single. 1. 4376±0. 0093.
   (4) When sire is a twin and paternal granddam is a twin. 1. 4003±0. 0082.
   (5) When either sire or paternal granddam is a triplet... 1. 520 ±0. 025.

TABLE X(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1–1, 1–2, 2–1, . . . of the sires and paternal granddams*

Sires and paternal granddams.	Offspring.		
	1	2	3
1–1...	62. 31	37. 09	0. 60
1–2...	62. 85	36. 75	. 40
2–1...	58. 00	40. 23	1. 77
2–2...	60. 45	39. 08	. 47
(*a*)...	49. 51	49. 02	1. 47
Based on total............................	60. 93	38. 30	. 77

a See footnote, Table I(B).

TABLE XI(A).—*Correlation between offspring and paternal granddams*

Paternal granddams.	Offspring.			Total.
	1	2	3	
1.....................................	2, 449	1, 551	43	4, 043
2.....................................	2, 165	1, 341	15	3, 521
3.....................................	79	58	1	138
Total...........................	4, 693	2, 950	59	7, 702

$$r = -0.0100 \pm 0.0084.$$

TABLE XI(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1, 2, and 3 of paternal granddams*

Paternal granddams.	Offspring.		
	1	2	3
1..	60. 57	38. 36	1. 07
2..	61. 49	38. 08	. 43
3..	57. 2	42. 0	. 8

Tables XII(A) and XII(B) represent the distribution of paternal grandsires and sires of the offspring treated in Tables II and IX. Here again we note that there is no significant difference whether paternal grandsires are singles or twins. This conclusion is further supported by obtaining from Table XIII(A) the correlation coefficient

$$r = -0.0147 \pm 0.0097.$$

We are thus unable to assert the existence of a significant correlation.

TABLE XII(A).—*Correlation between offspring and paternal grandsires*

Sires and paternal grandsires.	Offspring.			Total.
	1	2	3	
1–1	1, 160	632	11	1, 803
1–2	732	362	10	1, 104
2–1	564	423	6	993
2–2	465	278	13	756
1–3	8	2	....	10
3–1	9	7	2	18
2–3	18	9	3	30
3–2	....	....	....	....
3–3	....	4	....	4
Total	2, 956	1, 717	45	4, 718

Means of arrays:

    (1) When sire and paternal grandsire are singles........ 1. 3627±0. 0077.
    (2) When sire is single and paternal grandsire is a twin.. 1. 3460±0. 0099.
    (3) When sire is a twin and paternal grandsire is a single. 1. 4381±0. 011.
    (4) When sire is a twin and paternal grandsire is a twin. 1. 4021±0. 013.
    (5) When either sire or paternal grandsire is a triplet ... 1. 516 ±0. 054.

TABLE XII(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1–1, 1–2, 2–1, . . . of the sires and grandsires*

Sires and paternal grandsires.	Offspring.		
	1	2	3
1–1	64. 34	35. 05	0. 61
1–2	66. 30	32. 79	. 91
2–1	56. 80	42. 60	. 60
2–2	61. 51	36. 77	1. 72
(a)	56. 45	35. 49	8. 06
Based on total	62. 65	36. 39	. 96

a See note, Table I(B).

TABLE XIII(A).—*Correlation between offspring and paternal grandsires*

Paternal grandsires.	Offspring.			Total.
	1	2	3	
1	1, 733	1, 062	19	2, 814
2	1, 197	640	23	1, 860
3	26	15	3	44
Total	2, 956	1, 717	45	4, 718

$$r = -0.0147 \pm 0.0097.$$

TABLE XIII(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the paternal grandsires*

Paternal grandsires.	Offspring.		
	1	2	3
1	61. 58	37. 74	0. 68
2	64. 35	34. 41	1. 24
3	59. 09	34. 09	6. 82
Based on total...........................	62. 65	36. 39	. 96

Tables XIV(A) and XIV(B) represent the result of pooling the data on parents and offspring included in Tables II(A), VII(A), VIII(A), and IX(A).

TABLE XIV(A).—*Correlation between offspring and parents, obtained by combining into one table the data of Tables II(A), VII(A), VIII(A), and IX(A)*

Parents.	Offspring.			Total.
	1	2	3	
1..	12, 265	7, 575	134	19, 974
2..	8, 074	6, 124	153	14, 351
3..	191	190	14	395
Total...........................	20, 530	13, 889	301	34, 720

$$r = 0.0597 \pm 0.0036.$$

Means of arrays:
For parents... 1. 4361 ±0. 0020.
For offspring....................................... 1. 4174 ±0. 0020.

TABLE XIV(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the parents*

Parents.	Offspring.		
	1	2	3
1	61. 40	37. 92	0. 68
2	56. 27	42. 67	1. 06
3	48. 35	48. 10	3. 55
Based on total...........................	59. 13	40. 00	. 87

It may be noted that the percentage of recorded twins produced by twin parents is larger than the percentage of recorded twins produced by single parents. The correlation coefficient between sizes of litters in which parents and offspring are born is given by

$$r = 0.0597 \pm 0.0036.$$

It may also be noted that, on the whole, there is a slightly and significantly larger percentage of twins among parents than among recorded offspring. It must be remembered in this connection that the record of any parent is repeated a number of times equal to the number of the recorded offspring of the parent. Otherwise, one might be led to argue in a very obvious manner that if twinning is inherited and if there is no selection against twins, the percentage of twins in the offspring would exceed the percentage among parents, but such an argument is not valid when we repeat the parent, as above explained.

DATA NOT INCLUDED IN FOREGOING TABLES AND CALCULATIONS

Tables XV, XVI, and XVII exhibit data excluded from calculations of statistical constants because of the abnormal proportion of twins or singles recorded among the offspring of a single sire. The reasons and criteria for such exclusion are given under "Repetition of sires and paternal grandparents."

TABLE XV.—*Size of litter in which offspring are born and size of litter in which pairs of parents are born (from data excluded in making Tables I and IX)*

Sires and dams.	Offspring.			Total.
	1	2	3	
1–1	245	145	. . . .	390
1–2	150	160	3	313
2–1	238	63	. . . .	301
2–2	133	88	3	224
1–3	3	7	. . . .	10
3–1	. . . .	. . . .	. . . .	. . . .
2–3	1	2	. . . .	3
3–2	. . . .	. . . .	. . . .	. . . .
3–3	. . . .	. . . .	. . . .	. . . .
Total	770	465	6	1,241

TABLE XVI.—*Sizes of litters in which offspring and sires and paternal granddams are born (from data excluded in making Tables X and XI)*

Sires and paternal granddams.	Offspring.			Total.
	1	2	3	
1–1	197	168	. . . .	365
1–2	64	. . . .	. . . .	64
2–1	113	83	2	198
2–2	211	58	. . . .	269
Total	585	309	2	896

TABLE XVII.—*Sizes of litters in which offspring and sires and paternal grandsires are born (from data excluded in making Tables XII and XIII)*

Sires and paternal grandsires.	Offspring.			Total.
	1	3	3	
1–1	51	28	....	79
1–2	70	58	....	128
2–1	111	35	....	146
2–2	89	99	....	188
1–3	15	....	....	15
Total	336	220	....	556

DATA ON ANIMALS WITH A "COMPLETE AMERICAN" PEDIGREE

What we shall mean here by a "complete American" pedigree is a case where the offspring have parents and grandparents American-born, so that we have records as to whether these ancestors were born in states 1, 2, or 3. Statements are frequently made about the effects of climate on fertility; therefore we have thought that it would be of some interest to select from the total group of offspring that subgroup whose grandparents as well as parents are American-born. This requires that three generations at least be American born.[1] Further, the total subgroup of breeders who import a great deal may form a class that differs significantly in the handling of sheep from the totality of breeders who do not import.

This restriction to cases of "complete American" pedigree has brought our total number of cases down to only a little over one-third of the number treated in Tables I to XIV, but it seems worth while to present the tables showing corresponding frequencies of those with "complete American" pedigrees and to make some comparisons.

Any pedigree that has any grandparent not American-born is called an "incomplete" pedigree. It is, of course, incomplete in that we have no record as to whether a grandparent was born single, in twins, or in triplets.

TABLES FOR SIRES, DAMS, AND OFFSPRING WITH A "COMPLETE AMERICAN" PEDIGREE

Table XVIII(A) corresponds to Table I(A). Here we find for the correlation between the sum of numbers in litters in which sire and dam are born and the number in corresponding litters in which offspring are born, $r = 0.129 \pm 0.011$.

This coefficient is greater than that for the total population by 0.041, and this difference is just about three times its probable error. Hence, it is doubtful whether the difference is significant.

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[1] Wallace, A. R. Darwinism . . . p. 154. London, 1896.

TABLE XVIII(A).—*Correlation between offspring and pairs of parents with "complete American" pedigree*

Sires and dams.	Offspring.			Total.
	1	2	3	
1-1	825	368	8	1,201
1-2	562	357	6	925
2-1	492	282	1	775
2-2	338	275	13	626
1-3	3	11	....	14
3-1	5	7	....	12
2-3	6	8	3	17
3-2	3	3	3	9
3-3	....	....	....	....
Total	2,234	1,311	34	3,579

$$r = 0.129 \pm 0.011,$$

where r is the correlation coefficient between the sum of numbers in litters in which sire and dam are born and the number in corresponding litter in which offspring are born.

Means of arrays of offspring:

(1) When sire and dam are singles...................... 1.3197 ± 0.0094.
(2) When sire is a single and dam is a twin............. 1.399 ± 0.011.
(3) When sire is a twin and dam is a single............. 1.366 ± 0.012.
(4) When sire is a twin and dam is a twin.............. 1.481 ± 0.014.
(5) When either sire or dam is a triplet................ 1.788 ± 0.058.

TABLE XVIII(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to the states 1-1, 1-2, 2-1, . . . of the sires and dams*

Sires and dams.	Offspring.		
	1	2	3
1-1	68.69	30.64	0.67
1-2	60.75	38.59	.66
2-1	63.48	36.39	.13
2-2	53.99	43.93	2.08
(a)	32.69	55.77	11.54
Based on total	62.42	36.63	.95

a See footnote, Table I(B).

There is a slightly smaller proportion of twins among offspring of the "complete American" born than among the offspring of the total population, but the difference is only about three times its probable error, and there is some question as to its significance. The results obtained from means of arrays are not significantly different whether found from Tables I or XVIII.

TABLES FOR DAMS AND OFFSPRING WITH "COMPLETE AMERICAN" PEDIGREE

Table XIX(A) corresponds to Table II(A) but is limited to data from American-born grandparents. Here we have for the correlation of size of litter in which dams and offspring are born,

$$r = 0.128 \pm 0.010.$$

This coefficient is greater than that for the total population by 0.041, and this difference is slightly more than three times its probable error, but it still is far from certain that the difference is not to be attributed to random sampling.

TABLE XIX(A).—*Correlation between offspring and dams with "complete American" pedigree*

Dams.	Offspring.			Total.
	1	2	3	
1..	1, 505	709	9	2, 223
2..	985	704	24	1, 713
3..	10	21	3	34
Total..........................	2, 500	1, 434	36	3, 970

Means:
    Dams... 1. 4486±0. 0055.
    Offspring... 1. 3793±0. 0054.
Means of arrays:
    (1) When dam is a single............................. 1. 3270±0. 0068.
    (2) When dam is a twin............................... 1. 4390±0. 0082.
    (3) When dam is a triplet............................. 1. 794 ±0. 067.

$$r = 0.128 \pm 0.010.$$

TABLE XIX(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to the states 1, 2, and 3 of the dams*

Dams.	Offspring.		
	1	2	3
1..	67. 70	31. 89	0. 41
2..	57. 50	41. 10	1. 40
3..	29. 41	61. 76	8. 83
Based on total	62. 97	36. 12	. 91

TABLES FOR DAMS AND MATERNAL GRANDPARENTS WITH A "COMPLETE AMERICAN" PEDIGREE

Tables XX to XXV correspond to Tables III to VIII, and the results to be drawn from the tables based on data of the "complete American" pedigrees do not differ essentially from those drawn from the total population. Here again, as with the total population, there appears to be a slight correlation between size of litter in which maternal granddams and offspring are born, but we are unable to make a similar assertion about maternal grandsires and offspring.

TABLE XX(A).—*Correlation between offspring and maternal granddams with "complete American" pedigree*

Dams and maternal granddams.	Offspring.			Total.
	1	2	3	
1–1	946	432	6	1,384
1–2	543	271	3	817
2–1	530	362	9	901
2–2	450	333	15	798
1–3	16	6	....	22
3–1	4	6	2	12
2–3	5	9	....	14
3–2	5	10	1	16
3–3	1	5	....	6
Total	2,500	1,434	36	3,970

Means of arrays of offspring:

    (1) When the dam and granddam are singles........... 1. 3208 ±0. 0086.
    (2) When the dam is a single and the granddam a twin. 1. 339 ±0. 011.
    (3) When the dam is a twin and the granddam a single. 1. 412 ±0. 011.
    (4) When the dam is a twin and the granddam a twin.. 1. 455 ±0. 013.
    (5) When either dam or granddam is a triplet........... 1. 600 ±0. 046.

TABLE XX(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to the states 1–1, 1–2, 2–1, 2–2, ... of the dams and maternal granddams*

Dams and maternal granddams.	Offspring.		
	1	2	3
1–1	68. 35	31. 21	0. 44
1–2	66. 46	33. 17	. 37
2–1	58. 82	40. 18	1. 00
2–2	56. 39	41. 73	1. 88
(a)	44. 28	51. 43	4. 29
Based on totals	62. 97	36. 12	. 91

a See footnote, Table I (B).

TABLE XXI(A).—*Correlation between offspring and maternal grandsires with "complete American" pedigree*

Dams and maternal grandsires.	Offspring.			Total.
	1	2	3	
1–1	900	431	2	1,333
1–2	582	258	6	846
2–1	579	424	13	1,016
2–2	383	277	11	671
1–3	23	20	1	44
3–1	7	15	3	25
2–3	23	3	....	26
3–2	3	6	....	9
3–3	....	....	....	....
Total	2,500	1,434	36	3,970

TABLE XXI(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to the states 1–1, 1–2, 2–1, ... of the dams and maternal grandsires*

Dams and maternal grandsires.	Offspring.		
	1	2	3
1–1	67. 52	32. 33	0. 15
1–2	68. 79	30. 50	. 71
2–1	56. 99	41. 73	1. 28
2–2	57. 08	41. 28	1. 64
(a)	53. 85	42. 31	3. 84
Based on total	62. 97	36. 12	. 91

a See footnote, Table I (B).

TABLE XXII.—*Correlation between offspring and maternal granddams with "complete American" pedigree*

Maternal granddams.	Offspring.			Total.
	1	2	3	
1	1,480	800	17	2,297
2	998	614	19	1,631
3	22	20	....	42
Total	2,500	1,434	36	3,970

$r = 0.040 \pm 0.011$.

Means of arrays of offspring:

(1) When maternal granddams are singles............... 1. 3631 ± 0. 0070.
(2) When maternal granddams are twins............... 1. 3898 ± 0. 0087.
(3) When maternal granddams are triplets............. 1. 516 ± 0. 052.

TABLE XXIII.—*Correlation between offspring and maternal grandsires with "complete American" pedigree*

Maternal grandsires.	Offspring.			Total.
	1	2	3	
1	1,486	870	18	2,374
2	968	541	17	1,526
3	46	23	1	70
Total	2,500	1,434	36	3,970

$$r = -0.0068 \pm 0.011.$$

Means of arrays of offspring:

(1) When maternal grandsires are singles................ 1.3816 ± 0.0069.
(2) When maternal grandsires are twins................ 1.3768 ± 0.0090.
(3) When maternal grandsires are triplets.............. 1.357 ± 0.041.

TABLE XXIV(A).—*Correlation between dams and maternal granddams with "complete American" pedigree*

Maternal granddams.	Dams.			Total.
	1	2	3	
1......................................	1,175	725	11	1,911
2......................................	673	627	11	1,311
3......................................	18	11	4	33
Total...........................	1,866	1,363	26	3,255

$$r = 0.103 \pm 0.012.$$

TABLE XXIV(B).—*Percentages of dams in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the maternal granddams*

Maternal granddams.	Dams.		
	1	2	3
1	61.49	37.94	0.57
2	51.33	47.83	.84
3	54.55	33.33	12.12
Based on total...........................	57.33	41.87	.80

TABLE XXV(A).—*Correlation between dams and maternal grandsires with "complete American" pedigree*

Maternal grandsires.	Dams.			Total.
	1	2	3	
1............................	1, 111	808	19	1, 938
2............................	718	531	7	1, 256
3............................	37	24	....	61
Total.......................	1, 866	1, 363	26	3, 255

$r = 0.007 \pm 0.012$.

TABLE XXV(B).—*Percentages of dams in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the grandsires*

Grandsires.	Dams.		
	1	2	3
1....................................	57. 33	41. 69	0. 98
2....................................	57. 17	42. 28	. 55
3....................................	60. 66	39. 34	....
Based on total.......................	57. 33	41. 87	. 80

For the "complete American" subclass (Table XXV) we are also unable to assert a significant correlation between maternal grandsire and dams. This fact makes it appear still a little more probable that sex is in some way connected with the tendency of twins to produce twins. [Compare Tables VIII(A) and XXV(A).] This point will be further treated under "Analysis of data on parents and offspring separated with regard to sex."

TABLES FOR SIRES AND OFFSPRING WITH A "COMPLETE AMERICAN" PEDIGREE

While from the data of Table XXVI(A) the correlation coefficient, $r = 0.072 \pm 0.011$, is a little larger than for the total population, the difference may be ascribed to random sampling. Just as in the "Analysis of data for sires and offspring" we have found mean values of an index number for sires of different classes, so here we have the following values for mean index numbers for animals of "complete American" pedigree:

For sires born in singles ("complete American"), we have. 1. 296±0. 012.
For sires born in twins ("complete American"), we have. 1. 375±0. 017.
For remaining sires born in singles, we have.............. 1. 353±0. 010.
For remaining sires born in twins, we have............... 1. 384±0. 014.

TABLE XXVI(A).—*Correlation between offspring and sires with "complete American" pedigree*

Sires.	Offspring.			Total.
	1	2	3	
1	1,390	736	15	2,141
2	836	565	17	1,418
3	8	10	2	20
Total	2,234	1,311	34	3,579

$$r = 0.072 \pm 0.011$$

Means of arrays:

  (1) When sires are singles............................ 1.3578 ± 0.0072.
  (2) When sires are twins............................. 1.4224 ± 0.0093.
  (3) When sires are triplets........................... 1.700 ± 0.095.

TABLE XXVI(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to the states 1, 2, and 3 of the sires with "complete American" pedigree*

Sires.	Offspring.		
	1	2	3
1	64.92	34.38	0.70
2	58.96	39.84	1.20
3	40.0	50.0	10.0
Based on total.............................	62.42	36.63	.95

All our results with index numbers show consistently a larger relative production of twins by twin sires than by single sires. It may be noted, however, that in the case of the subclass remaining after those of "complete American" pedigree are removed, the difference is not three times its probable error. This surely means that it requires very large numbers to establish with any considerable certainty the significance of the difference.

TABLES FOR SIRES AND PATERNAL GRANDPARENTS

Tables XXVII to XXX for "complete American" pedigrees correspond to Tables X to XIII for the total population. Here again, as with the total population, we are unable to assert the existence of a significant correlation between paternal grandparents and offspring with respect to being born in singles or in twins.

TABLE XXVII(A).—*Correlation between offspring and paternal granddams with "complete American" pedigree*

Sires and paternal granddams.	Offspring.			Total.
	1	2	3	
1-1	750	415	8	1,173
1-2	619	303	6	928
2-1	401	297	11	709
2-2	421	265	6	692
1-3	21	19	1	41
3-1	7	5	2	14
2-3	14	2	. . . .	16
3-2	1	5	. . . .	6
3-3	. . . .	. . . .	. . . .	. . . .
Total	2,234	1,311	34	3,579

TABLE XXVII(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to the states 1-1, 1-2, 2-1, . . . of the sires and paternal granddams with "complete American" pedigree*

Sires and paternal granddams.	Offspring.		
	1	2	3
1-1	63.94	35.38	0.68
1-2	66.70	32.65	.65
2-1	56.56	41.89	1.55
2-2	60.84	38.30	.86
(a)	55.84	40.26	3.89
Based on total	62.42	36.63	.95

[a] See footnote, Table I(B).

TABLE XXVIII.—*Correlation between offspring and paternal granddams with "complete American" pedigree*

Paternal granddams.	Offspring.			Total.
	1	2	3	
1	1,158	717	21	1,896
2	1,041	573	12	1,626
3	35	21	1	57
Total	2,234	1,311	34	3,579

$r = -0.028 \pm 0.011.$

TABLE XXIX(A).—*Correlation between offspring and paternal grandsires with "complete American" pedigree*

Sires and paternal grandsires.	Offspring.			Total.
	1	2	3	
1–1....................................	869	466	5	1,340
1–2....................................	520	270	10	800
2–1....................................	450	342	6	798
2–2....................................	369	216	8	593
1–3....................................	1	....	....	1
3–1....................................	8	6	2	16
2–3....................................	17	7	3	27
3–2....................................	....	....	....	....
3–3....................................	....	4	....	4
Total........................	2,234	1,311	34	3,579

TABLE XXIX(B).—*Percentage of offspring in states 1, 2, and 3 to correspond to the states 1–1, 1–2, 2–1, . . . of the sires and paternal grandsires with "complete American" pedigree*

Sires and paternal grandsires.	Offspring.		
	1	2	3
1–1..	64.85	34.78	0.37
1–2..	65.00	33.75	1.25
2–1..	56.39	42.86	.75
2–2..	62.23	36.42	1.35
(a)..	54.17	35.42	10.41
Based on total............................	62.42	36.63	.95

a See note, Table I(B).

TABLE XXX.—*Correlation between offspring and paternal grandsires with "complete American" pedigree*

Paternal grandsires.	Offspring.			Total.
	1	2	3	
1...............................	1,327	814	13	2,154
2...............................	889	486	18	1,393
3...............................	18	11	3	32
Total.......................	2,234	1,311	34	3,579

$r = -0.0059 \pm 0.011$.

Tables XXXV and XXXVI exhibit data of the "complete American" class excluded under the criteria of "Repetition of sires and paternal grandparents."

ANALYSIS OF DATA ON PARENTS AND OFFSPRING SEPARATED WITH REGARD TO SEX

Table XXXI exhibits data for the correlation of size of litters in which sires are born and sizes of litters in which their female offspring are born. The importance of investigating the tendency of twin sires to produce a larger percentage of twins among female offspring than single sires produce among female offspring is suggested by the fact that, from the data of Tables VIII and XXV, we were unable to assert the existence of a significant correlation.

TABLE XXXI(A).—*Correlation between female offspring and sires*

Sires.	Female offspring.			Total.
	1	2	3	
1	2, 373	1, 388	11	3, 772
2	1, 440	930	19	2, 389
3	15	27	1	43
Total	3, 828	2, 345	31	6, 204

$r = 0.0410 \pm 0.0086.$

Means:

For sires .... $1.3987 \pm 0.0044.$

For female offspring .... $1.3880 \pm 0.0043.$

TABLE XXXI(B).—*Percentages of female offspring in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the sires*

Sires.	Female offspring.		
	1	2	3
1	62. 91	36. 80	0. 29
2	60. 28	38. 93	. 79
3	34. 88	62. 79	2. 33
Based on total	61. 70	37. 80	. 50

From Table XXXI(A) we obtain for the correlation

$$r = 0.0410 \pm 0.0086.$$

While this is a very small correlation, it is 4.6 times its probable error. While it is by no means finally established, it seems somewhat more probable that the values in Tables VIII and XXV are accidentally small under random sampling than that the correlation just given is insignificant. What is clear is that if any correlation exists, such correlation is so small that it requires immense numbers to establish its significance.

In order to eliminate the possible effects of the repetitions of grandsires, we shall investigate further the cases of maternal grandsires and dams by means of index numbers, such as are explained in the sections on "Analysis of data for sires and offspring" and "Tables for sires and offspring." The results with such index numbers may be stated as follows:

> For maternal grandsires born in singles, we have .... 1. 4462±0. 0073.
> For maternal grandsires born in twins, we have .... 1. 4574±0. 0093.

While the mean index number for twin maternal grandsires is thus a little larger than for single maternal grandsires, the difference is not large enough to enable us to assert its significance, when compared to fluctuations in sampling.

Tables XXXII to XXXIV are correlation tables for sizes of litters in which sires and male offspring, dams and male offspring, and dams and female offspring are born. In each of these cases we find significant correlations. The differences of these correlations are so small that we are unable to assert that the differences are significant. The values of these correlation coefficients are:

> For sires and male offspring .... r=0. 073±0. 012.
> For dams and male offspring .... r=0. 075±0. 011.
> For dams and female offspring .... r=0. 0925±0. 0080.

TABLE XXXII(A).—*Correlation between male offspring and sires*

Sires.	Male offspring.			Total.
	1	2	3	
1........................	1, 099	663	14	1, 776
2........................	724	549	18	1, 291
3........................	7	12	1	20
Total...............	1, 830	1, 224	33	3, 087

Means:
> For sires.. 1. 4310±0. 0066.
> For male offspring................................. 1. 4180±0. 0067.

TABLE XXXII(B).—*Percentages of the male offspring in states 1, 2, and 3 to correspond to the states 1, 2, and 3 of the sires*

Sires.	Male offspring.		
	1	2	3
1........................	61. 88	37. 33	0. 79
2........................	56. 09	42. 53	1. 38
3........................	35. 00	60. 00	5. 00
Based on total...............	59. 28	39. 65	1. 07

TABLE XXXIII(A).—*Correlation between male offspring and dams*

Dams.	Male offspring.			Total.
	1	2	3	
1	1, 195	704	12	1, 911
2	861	657	19	1, 537
3	26	20	3	49
Total	2, 082	1, 381	34	3, 497

TABLE XXXIII(B).—*Percentages of male offspring in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the dams*

Dams.	Male offspring.		
	1	2	3
1	62. 53	36. 84	0. 63
2	56. 02	42. 75	1. 23
3	53. 06	40. 82	6. 12

TABLE XXXIV(A).—*Correlation between female offspring and dams*

Dams.	Female offspring.			Total.
	1	2	3	
1	2, 589	1, 342	15	3, 946
2	1, 725	1, 273	21	3, 019
3	32	38	. . . .	70
Total	4, 346	2, 653	36	7, 035

TABLE XXXIV(B).—*Percentages of female offspring in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the dams*

Dams.	Female offspring.		
	1	2	3
1	65. 61	34. 01	0. 38
2	57. 14	42. 17	. 69
3	45. 71	54. 29	. . . .
Based on total	61. 78	37. 71	. 51

Tables XXXV and XXXVI exhibit data excluded, by the criteria of the section on "Repetition of sires and paternal grandparents," from the "complete American" subgroup.

TABLE XXXV.—*Sizes of litters in which offspring and pairs of parents are born, "complete American" (from data excluded in making Table XVIII)*

Sires and dams.	Offspring.			Total.
	1	2	3	
1–1	63			63
1–2	18			18
2–1	120	52		172
2–2	64	69	2	135
1–3				
3–1				
2–3	1	2		3
3–2				
3–3				
Total	266	123	2	391

TABLE XXXVI.—*Sizes of litters in which offspring and sires are born (from data excluded in making Table XXVI)*

Sires.	Offspring.			Total.
	1	2	3	
1	81			81
2	185	123	2	310
3				
Total	266	123	2	391

GENERAL CONCLUSIONS

(1) For the class of sheep considered in this investigation we find that, in general, the twin parents give a larger percentage of twins among offspring than do parents born as singles. See Tables I(B), II(B), VII(B), IX(B), and XIV(B).

(2) The small positive correlation coefficient between the sum of numbers in litters in which the two parents are born and the size of litter in which the corresponding offspring are born is significant. The value of the coefficient is in each case more than 11 times the probable error. See results derived from Tables I(A) and XVIII(A).

(3) The small positive correlation coefficients between sizes of litters in which dams are born and sizes of litters in which their offspring are born are decidedly significant when judged by probable errors. See

results derived from Tables II(A), VII(A), XXIV(A), XXXIII(A), and XXXIV(A).

(4) There appears to be a small but significant correlation between sizes of litters in which sires are born and sizes of litters in which their offspring are born. It seems probable that this correlation should be attributed almost entirely, if not wholly, to correlation between sires and male offspring. The correlations seem to differ with the sexes. The correlation coefficients for sires and female offspring are so small that their significance is much in doubt even with the large numbers we have used. See results derived from Tables VIII(A), IX(A), XXV(A), XXVI(A), XXXI(A), and XXXII(A).

(5) There appears to be a significant correlation between maternal granddams and offspring, but we are unable to assert any significant correlation for the other grandparents and offspring. It would surely require immense numbers to establish the significance of such correlation, if it exists.

(6) The means of arrays show the small but general tendency of either or both twin parents and twin maternal granddams to produce a larger proportion of twins than are produced when the corresponding individuals in the ancestry are singles.

(7) As it requires large numbers to establish the significance of the differences which we have found, it should not be surprising if within a flock of fair size, say, a flock of 100, one may in some cases get even a larger percentage of twins from single parents than from twin parents. Such fluctuations should be expected to occur occasionally in taking a random sample of no more than 100 individuals, even if in the long run twins tend to produce a somewhat higher percentage of twins than do singles.

SOIL PROTOZOA[1]

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I.—METHOD FOR COUNTING PROTOZOA

INTRODUCTION

Repeated claims that soil protozoa are detrimental to other soil micro-organisms have led soil biologists to begin the study of soil protozoology in order to determine, if possible, to what extent these organisms influence soil fertility.[2]

In order to facilitate the counting and examination of protozoa, several investigators have cultivated these organisms in artificial culture solutions. Goodey (7) studied the protozoa which were developed in soil extract and dilute hay infusion, but made no attempt to count them. Likewise Martin and Lewin (13) made careful examinations of the organisms which they cultivated in an infusion of horse manure. Rahn (15) employed peptone and sugar solutions, thus allowing the organisms to develop in a culture solution of 1 to 100 dilution for from 7 to 14 days, after which an aliquot of the solution was examined for protozoa. Killer (10) used the dilution method in order to determine the approximate numbers of soil protozoa that were developed in Giltay's, mannite, and peptone solutions. Francé (6) studied soil protozoa developed in artificial culture solutions and enumerated the organisms from the various soils examined by mixing an average sample of the solution with water and then examining the resulting solution drop by drop. Likewise Cauda and Sangiorgi (2) employed Giltay's, Omelianski's, Hiltner's, peptone, and mannite solutions and determined the numbers of organisms developed by dilution and direct count. In order to find the best culture solution for protozoan development, Cunningham and Löhnis (4) grew these organisms in many different solutions, and later Cunningham employed soil extract and blood-meal extract. The last-named investigator (3) also used the dilution method for the enumeration of the organisms. The results obtained with the dilution method by these investigators have been somewhat irregular. The irregularity in the results secured by the application of this method is shown quite clearly by Cunningham (3). The same irregularity has been experienced by the writer, who found the error to be several hundred per cent in many cases.

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IMPROVED LOOP METHOD

Because of the inadequacy of the methods heretofore used, the loop method which was employed by Müller (14) for counting bacteria was improved upon. The improved method proved much more accurate and required but a very small amount of manipulation.

A platinum wire was bent into a permanent loop. The quantity of solution that could be transferred by means of this loop was then determined by carefully weighing films of the culture solution on a sensitive analytical balance. The average of several weights was taken and the quantity of liquid that could be transferred by the loop was calculated into cubic centimeters. To facilitate the counting of the organisms on the slide, a quarter of an inch square in the center of an ordinary glass slide was carefully ruled into 60 to 80 small squares by means of a sharp quartz crystal. A film of the culture solution containing the living protozoa was then transferred to the ruled area on the clean glass slide. In this manner the living protozoa were counted with the low power of the microscope, and from the number of organisms transferred in the loop the numbers per cubic centimeter were calculated.

The platinum loop was slightly bent, making an angle of 30° to 35°, so as to facilitate touching the slide in the same manner at each transfer and to prevent the draining of the solution which would adhere to the support of the loop.

In making the counts the platinum loop was first sterilized in a flame, and every precaution ordinarily observed in bacteriological work was taken. The protozoa of not less than three loops of culture solution were counted, and the average of the several counts was recorded. It was found by experience that not more than 300 to 400 protozoa could be counted in a film which had been transferred by a loop of 0.0020 to 0.0025 gm. transference capacity, as the culture solution on the slide would be evaporated before all the organisms could be counted. Therefore, two platinum loops, one of 0.0020 to 0.0025 gm. transference capacity and the other of 0.001 gm., were employed. When the number of organisms became so great that they could not all be counted in the large film, the loop of smaller capacity was employed. Where the organisms numbered more than 300 for the small loop, the film of culture solution containing the protozoa was transferred to a specially prepared slide. This glass slide has a square cell in the center (3.7 by 3.7 mm.), the capacity of which is about 0.002 gm. of water at 22° C. (when the readings are made the cell is almost completely filled with culture solution, thus reducing the possibility of error due to capillarity). The surface within the cell is accurately ruled into 25 large divisions, and one of the large divisions again ruled into 25 small fields. The film of solution containing the organisms was carefully spread over the entire surface and a cover slip laid over the cell, thus preventing evaporation. The organisms

in several of the large fields were counted, and the average of these figures was multiplied by 25. The number obtained was then multiplied by the standard number of the platinum loop. With the average of several of such counts and calculations, the number of organisms was determined. In case the organisms were too small to be readily seen by means of the low power of the microscope and too numerous to be calculated from the large divisions of the slide, the approximate number could be obtained by counting the organisms in the smaller divisions.

When many very active flagellates were studied, the latter method of counting the organisms in several fields proved superior to the complete counting method, on account of the fact that the manipulator might count one organism several times. But, as a general rule, in every case where the organisms were not too active and where the organisms numbered from 300 to 400 in the solution transferred by the large loop and 300 by the small loop the direct count of all the organisms transferred by the loop was made, in order that the error incurred be the minimum.

In computing the organisms on the slide several fields in different positions were counted and averaged, in this way eliminating as much as possible the very small error which might be incurred, owing to the very slight capillarity.

The flagellates were always counted while active, as otherwise in many cases they could not be distinguished from cysts. In case of the ciliates, however, the organisms could be very easily distinguished when inactive. Thus, in counting the ciliates, the film of solution was first passed through the fumes of a 1 per cent osmic-acid solution, as suggested by Goodey (7). When many ciliates were present in a solution containing numerous flagellates, the counts of the living flagellates were made first, and then several other films of solution treated with osmic acid were examined and the number of ciliates determined.

In order to determine the variation in the amount of solutions which a platinum loop would transfer, two loops of different size prepared for this purpose were tested by weighing films of different culture solutions. The weights recorded below were made by placing the film of the solution upon a watch glass, which was counterpoised by another of the same size. The rider was placed at 0.1 mg. and the correct addition of weight calculated by oscillations. The temperature of the room was 22° C. and of the balance case 22.5° when the weights were made. (See Tables I–V.)

TABLE I.—*Variation in transference capacity of a platinum loop when used for several solutions and the variation after it had been used for a period of time* [a]

[Weight of solution transferred]

Distilled water at 22° C. when loop was first used.	Distilled water at 22° C. after loop used 45 days.	Distilled water at 5° C.	Hay infusion at 22° C.	Blood extract at 22° C.	Soil extract at 22° C.
Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
0. 00200	0. 00199	0. 00150	0. 00186	0. 00194	0. 00170
. 00199	. 00199	. 00149	. 00185	. 00191	. 00169
. 00199	. 00200	. 00149	. 00184	. 00193	. 00169
. 00200	. 00198	. 00149	. 00184	. 00192	. 00171
. 00199	. 00198	. 00150	. 00183	. 00193	. 00170
. 00199	. 00198	. 00150	. 00183	. 00192	. . . .
[b]. 001993	[b]. 001987	[b]. 001445	[b]. 001841	[b]. 001925	[b]. 001698

[a] 1 gm. of distilled water at 22° C.=1 c. c.   [b] Average.

TABLE II.—*Density of culture solutions compared with water at the same temperature*

Temperature.	Water.	Hay infusion.	Blood extract.
° *C.*			
6	0. 999970	0. 999040	0. 998885
22	. 997828	. 997460	. 997648

TABLE III.—*Experimental error when all the organisms of the loop numbering about 9,000 per cubic centimeter are counted under the low power of the microscope*

Number of films.	Number of organisms in film.	Deviation.	Deviation squared.
1.	14	5. 2	27. 0
2.	22	2. 8	7. 8
3.	13	6. 2	38. 4
4.	14	5. 2	27. 0
5.	23	3. 8	14. 4
6.	19	. 2	. 4
7.	26	6. 8	46. 2
8.	28	8. 8	77. 4
9.	23	3. 8	14. 4
10. . . . . :	10	9. 2	84. 6
Sum.	192. 0	. . . .	337. 6
Mean.	19. 2	. . . .	. . . .

$$\text{Probable error} = \pm 0.6745 \sqrt{\frac{\Sigma D^2}{n(n-1)}}$$

$$= \pm 0.6745 \sqrt{\frac{337.6}{90}} = 1.92.$$

$$= \pm 0.6745 \times 1.92 = 1.29.$$

Percentage of error = 1.29 ÷ 19.2 = 6.74.

TABLE IV.—*Experimental error when all the organisms of the loop numbering about 140,000 per cubic centimeter are counted under the low power of the microscope*

Number of films.	Number of organisms in film.	Deviation.	Deviation squared.
1...	263	22. 5	506. 0
2...	251	34. 5	1, 190. 0
3...	286	. 5	. 2
4...	377	91. 5	8, 370. 0
5...	266	19. 5	381. 0
6...	290	4. 5	22. 1
7...	302	16. 5	72. 0
8...	223	62. 5	3, 910. 0
9...	326	40. 5	1, 640. 0
10...	266	19. 5	381. 0
Sum..	2, 850	....	16, 672. 3
Mean.......................................	285	....	....

Probable error $= \pm 0.6745 \sqrt{\dfrac{16,672.3}{90}} = 13.62.$

$\qquad = \pm 0.6745 \times 13.62 = 9.20.$

Percentage of error $= 9.20 \div 285.0 = 3.23.$

TABLE V.—*Probable error when the organisms of several fields are counted and when the number totals about 450,000 per cubic centimeter*

Number of films.	Number of organisms.					Average.	Deviation.	Deviation squared.
	Field No. 1.	Field No. 2.	Field No. 3.	Field No. 4.	Field No. 5.			
1....................	56	30	24	30	22	32. 4	4. 2	17. 6
2....................	40	10	35	31	23	27. 8	8. 8	77. 5
3....................	20	15	18	25	23	16. 2	20. 4	416. 0
4....................	23′	24	28	25	32	26. 4	10. 2	104. 0
5....................	26	37	29	53	45	38. 0	1. 4	1. 9
6....................	36	48	53	51	62	50. 0	13. 4	179. 8
7....................	26	31	32	26	19	26. 8	9. 8	96. 0
8....................	33	52	42	60	69	51. 2	14. 6	213. 2
9....................	61	64	57	52	49	56. 6	20. 0	400. 0
10....................	35	36	59	31	44	41. 0	4. 4	19. 3
Sum...........	......	......	......	......	......	366. 4	....	1, 525. 3
Mean...........	......	......	......	......	......	36. 6	....	....

Probable error $= \pm 0.6745 \sqrt{\dfrac{1,525.3}{90}} = 4.15.$

$\qquad = \pm 0.6745 \times 4.15 = 2.799.$

Percentage of error $= 2.799 \div 36.6 = 7.64.$

The average of the experimental error incurred in counting is therefore 5.87 per cent.

EFFICIENCY OF THE METHOD

Upon examining the foregoing data, it is seen that the variation in weights of successive amounts transferred from a solution at a given temperature is very slight, the greatest variation being not more than 1 per cent.

In applying the improved loop method all calculations are based upon distilled water at 22° C., or room temperature, as a standard. Hence, a correction must be made when solutions of different surface tension are employed, as the amount of culture solution transferred by the standard loop would vary with the liquid. This is noted in Table I in comparing the amount of distilled water, hay infusion, blood extract, and soil extract transferred with the same loop.

In order to facilitate calculations, 1 gm. of distilled water at 22° C. was used as the standard to represent 1 c. c.

Upon examining Table II, it is noted that the error incurred on account of the variation in the density of solutions used is practically negligible.

Where the number of organisms per cubic centimeter is relatively small, the experimental error incurred in counting all the organisms contained in the loop is 6.74 per cent, as seen from Table III. As shown in Table IV, when the number of organisms per cubic centimeter is greater and all the organisms in the loop are counted, the error is smaller. In counting relatively small numbers of organisms in several fields of a specially ruled slide the experimental error is greater in proportion than if the organisms of the entire loop are counted, as shown in Table V.

SUMMARY OF PART I

(1) While the improved loop method is by no means devoid of errors, it has proved much more satisfactory than any of the other above-mentioned methods.

(2) It makes possible the quantitative study of the development of organisms in solutions without greatly altering the culture solutions.

(3) It is comparatively simple and requires but little time for any single determination.

(4) The improved loop method requires additional calculation because corrections must be made when culture solutions of different surface tension than distilled water at 22° are employed, which is not the case when the volume is always constant.

(5) The average experimental error is about 7 per cent.

II.—PROTOZOA OF GREENHOUSE SOILS

INTRODUCTION

That small organisms other than bacteria, fungi, algæ, and worms— i. e., protozoa—exist in rich soils was known by Ehrenberg (5) as far back as 1837. Greef (9) in 1866 recorded the presence of very large living

forms in the soil, but it was probably not until the work of Breal (1) in 1896, later by Francé (6), and by Wolff (19) in 1909, that mention was made of the probable effect which protozoa might have upon soil fertility due to the destructive influence which they might have upon other soil micro-organisms. Then again, the very extensive work on soil sterilization by Russell and Hutchinson (16, 17) in 1909 and 1913 has been carried out for the purpose of showing the effect of heat and antiseptics upon a detrimental factor in the soil, which those authors believe to be protozoa. In 1911 Goodey (7) describes the isolation of various protozoa from the soil and concludes that protozoa are inactive in normal soils. A few years later, however, he (8) concluded that the ciliated forms are present in the soil in the encysted condition and that the amœbæ and flagellates act as limiting factors. Martin (12), examining freshly collected soil for protozoa, concluded that the prevalence of protozoa in culture solutions was no indication of their presence in the living state in the soil. Upon the examination of cucumber-sick soil, Martin and Lewin (13) found eight different kinds of protozoa. Amœbæ were probably the dominant forms in the soil during examination. Flagellates were very rare. These investigators state that in absolutely saturated soils the ciliates may play an active part as a bacteria check but that "it is difficult to believe that they can exercise an important rôle in a sick soil-bed." Russell and Petherbridge (18) are also of the opinion that the factor which keeps down the numbers of bacteria in cucumber- and tomato-sick soil is biological. Killer (10), Rahn (15), Cunningham and Löhnis (4, p. 600), and later Cunningham (3) grew soil protozoa in various culture solutions and noted a great difference in the development of these organisms in artificial media.

Cunningham (3, p. 22) concludes that "the results given in this section prove conclusively that the soil protozoa, in solution at all events, exercise a very decided limiting effect on the numbers of bacteria."

DEVELOPMENT OF PROTOZOA IN ARTIFICIAL CULTURE SOLUTIONS

In this study it was the object of the writer:

(1) To compare the difference in numbers and species of protozoa developed in different culture solutions.

(2) To compare the protozoan development from varying amounts of soil.

(3) To compare the protozoan development from moist and dry soil.

(4) To compare the protozoan development from different greenhouse soils.

A large sample of clayey soil having a moisture content of 25 per cent and upon which alfalfa was grown in 1914 and then composted to a 20 per cent manure, was collected from a compost bin in the greenhouse. A portion of this soil was dried for three days at 35° C. Into 200 c. c.

Jena Erlenmeyer flasks were placed 100 c. c. portions of a 3 per cent dried-blood extract + 0.05 per cent of dibasic potassium phosphate (K_2HPO_4) prepared by boiling 30 gm. of dried blood with 1,000 c. c. of tap water for one hour and then adding 0.05 per cent of K_2HPO_4. In like manner 100 c. c. portions of Löhnis's (11, p. 118) soil extract with the addition of 0.05 per cent of dibasic potassium phosphate were put into another series of flasks. These flasks were plugged with cotton and sterilized in an autoclave and all the precautions taken as in bacteriological work. They were then carefully inoculated with 1, 2, 3, 5, 10, 20, 50, and 100 gm. portions of moist and dry soils. The solutions were examined under the microscope for living protozoa by carefully transferring a film of the culture solution to a clean glass slide. The inoculated flasks were then placed in a constant-temperature incubator and incubated at 22° C. Daily examinations at the same hour for a period of 30 days were made and the different types of protozoa enumerated by the improved loop method described on p. 512.

In order to compare the development of protozoa of different greenhouse soils, three other compost soils were collected:

(1) A 10 per cent manure and 10 per cent sand mixture upon which roses were grown in the greenhouse the previous year. At the time of collection this soil was exposed to the weather and contained 21.1 per cent of moisture.

(2) A 50 per cent manure mixture, which was also exposed to the weather and had a moisture content of 30.3 per cent. Roses were grown upon this soil the previous year.

(3) A 30 per cent compost which was planted to soy beans and corn seedlings. This was a dry soil, having a moisture content of 14.9 per cent.

Erlenmeyer flasks of 200 c. c. capacity containing 100 c. c. of the same extracts of dried blood and soil, as described above, were inoculated with 1, 20, 50, and 100 gm. portions of the four composts. These solutions were examined for protozoa and then incubated at 22° C. for eight days. At the same hour each day examinations and counts of the different types of protozoa were made.

The classification of the protozoa which was followed throughout this problem was as follows:

The small ciliates included all organisms from the smallest to and including *Colpidium colpoda* Ehrb. The vorticellæ were also included.

The large ciliates included all forms larger than *Colpidium colpoda*.

The flagellates included all the forms that were observed.

It was found at the outset that there were many minute organisms that could not be recognized easily under the low power, but which were easily seen with the high power of the microscope. Even under the high power it was impossible to distinguish between large bacteria and

what might appear to be protozoa. For this reason all of the protozoan counts were made under the low power. In order that the results might be concordant, every culture was examined about the same hour each day, for there is a great variation in the numbers of active organisms present at different hours during the day (Table VI). This is especially true for the first 10 to 15 days after inoculation.

TABLE VI.—*Variation per gram of soil in numbers of protozoa present in culture solutions at different hours of the same day*

Culture No.	Second day after inoculation.		Third day after inoculation.	
	9 a. m.	4 p. m.	9 a. m.	4 p. m.
721..........................	3, 272, 000	5, 430, 000	16, 800, 000	9, 982, 000
722..........................	6, 180, 000	7, 220, 000	6, 980, 000	5, 950, 000
723..........................	505, 000	1, 117, 000	798, 000	610, 000
724..........................	221, 400	585, 000	516, 000	221, 400

DEVELOPMENT OF PROTOZOA IN DIFFERENT CULTURE SOLUTIONS INOCU-LATED WITH VARYING AMOUNTS OF MOIST AND DRY SOIL

SMALL CILIATES.—The development of protozoa in the various solutions was quite different (as shown in Table VII). In comparing the development of the small ciliates in different media, it is apparent that the maximum development of these organisms occurred sooner in dried blood than in soil extract. In all inoculations of the dried-blood extract the greatest numbers were present on the third to the fourth day; the numbers then decreased, so that after the ninth day there were very few present throughout the remaining period of observation. In soil-extract solutions inoculated with the largest amounts of soil, the maximum development was from the third to the fourth day, whereas in solutions of the same extract inoculated with 1 gm. of dry soil they did not appear until the ninth day and on the sixteenth day with the same amount of moist soil. In both culture solutions the numbers of small ciliates developed were very small as compared with the number of flagellates. For the existence of small ciliates the soil extract seemed to be more favorable than dried blood. In comparing the numbers of organisms developed in the different solutions it was found that those which were inoculated with the largest amounts of soil did not show greater development than the ones to which the smallest amounts of soil had been added. Hence, on the gram basis more than a hundred times as many small ciliates developed from 1 gm. of soil as from 100 gm. It is noted that in nearly all inoculations of both media a greater number of organisms developed at an earlier period from the moist than from the dry soil. This difference was, however, not very great.

TABLE VII.—*Comparison of the numbers of small ciliates, large ciliates, and flagellates developed daily in different artificial culture solutions from varying amounts of moist and dry soil for a period of 30 days. These numbers are calculated to 1 gm. of dry soil*

Solution No.	Quantity of soil	Kind of soil	Medium	Kind of organisms	\	\	\	\	Days after inoculation.	\	\	\	\	\
	Gm.				1	2	3	4	5	6	7	8	9	10
10	1	Moist	Blood extract.	Small ciliates.			82,500	618,000			165,000		82,500	
				Large ciliates.		1,800,000	23,180,000	82,500	82,500	82,500	82,500		82,500	163,000
				Flagellates.										
11	3	...do...	...do...	Small ciliates.	26,800		53,600	134,000	26,800	26,800			26,800	26,800
				Large ciliates.			26,800	53,600						
				Flagellates.		684,000	853,000				16,500	413,000	412,000	253,000
12	5	...do...	...do...	Small ciliates.		115,200	33,000	132,000		16,500	16,500	33,000	132,000	99,000
				Large ciliates.			372,000	33,000		8,250	165,000	229,000	8,250	
				Flagellates.			24,700			66,000	8,250	8,250		
13	10	...do...	...do...	Small ciliates.		100,000	118,400	8,250	49,500			8,250	49,500	
				Large ciliates.	8,250		20,600	16,500		20,600	8,250			41,200
				Flagellates.										
14	20	...do...	...do...	Small ciliates.			4,120	4,120				4,120		
				Large ciliates.		8,250	37,120	16,500		16,500	28,800	8,250	12,400	24,700
				Flagellates.		166,000	1,650	3,300		3,300		1,650		
15	50	...do...	...do...	Small ciliates.			37,000				4,950	4,950	4,950	
				Large ciliates.			825							
				Flagellates.		113,200		1,650		1,650				
16	100	...do...	...do...	Small ciliates.	825	825						825		
				Large ciliates.	825	825						825		
				Flagellates.		134,000	5,970	2,470	825		2,470	3,300		6,600
20	1	Dry	...do...	Small ciliates.			61,800	61,800	61,800	309,000	127,000	61,800	61,700	61,700
				Large ciliates.			704,000	1,063,000	258,000	61,800	61,800	61,800	185,000	432,000
				Flagellates.		447,000	20,600	82,500		126,300		247,300		
21	3	...do...	...do...	Small ciliates.			20,600			20,600			26,000	26,000
				Large ciliates.										
				Flagellates.		170,000	3,267,000	3,520,000	82,500		82,500	61,800		
22	5	...do...	...do...	Small ciliates.		12,380	27,000	27,000	12,380	12,380	12,380	12,380	12,400	41,200
				Large ciliates.		61,700	1,625,000			12,380				
				Flagellates.			12,360		27,000	27,000		37,500		37,000
23	10	...do...	...do...	Small ciliates.			6,180	6,180	6,180		6,180	30,900	6,180	12,360
				Large ciliates.			307,000					18,550		
				Flagellates.		18,550	6,180	12,360	6,180				12,360	
24	20	...do...	...do...	Small ciliates.			123,600	101,970	3,090	3,090	3,090	30,900	3,090	18,540
				Large ciliates.			2,480	1,240						
				Flagellates.		12,360								
25	50	...do...	...do...	Small ciliates.		18,530	13,400	33,000	2,480		1,240	1,240	1,240	3,700
				Large ciliates.										
				Flagellates.			3,710							

No.	%	Condition	Medium	Organism									
26	100	...do...	...do...	Small ciliates									
				Large ciliates	618				618			618	618
				Flagellates	57,600	24,720	25,700		1,860		1,270	618	
30	1	Moist	Soil extract	Small ciliates				18,350	29,800		29,800	183,000	3,900,000
				Large ciliates				1,470,000			29,800		
				Flagellates			9,170						
31	3	...do...	...do...	Small ciliates		27,500	55,200	1,238,000	18,350	204,500	62,000	206,000	124,000
				Large ciliates				4,570	688,000		36,700	55,000	18,300
				Flagellates			9,170	4,570	5,170				
32	5	...do...	...do...	Small ciliates			705,000		36,700	18,350	73,500	18,350	18,300
				Large ciliates			18,300			183,500	9,170	18,300	9,150
				Flagellates						9,170			
33	10	...do...	...do...	Small ciliates			4,570	2,860	4,570	55,000	27,500	18,300	
				Large ciliates			157,200		4,570		9,170		
				Flagellates					5,590				
34	20	...do...	...do...	Small ciliates	4,580	51,600	1,830	4,570	3,670	4,570	4,570	4,570	45,800
				Large ciliates		3,670	5,590	13,700	918	13,700		18,300	
				Flagellates		1,830	918	1,830		1,830			
35	50	...do...	...do...	Small ciliates	12,600	57,300	1,830	9,180	2,860	9,180	1,830	1,830	13,750
				Large ciliates		3,670	1,830	918		918	918	19,500	
				Flagellates		918	918						
36	100	...do...	...do...	Small ciliates	1,830	918	2,860	2,860		2,860	2,860	918	10,400
				Large ciliates	24,020	20,620	2,860					918	9,186
				Flagellates								6,420	
40	1	Dry	...do...	Small ciliates				68,700	45,700			68,500	
				Large ciliates									
				Flagellates									
41	3	...do...	...do...	Small ciliates			13,750	6,800,000	359,000	271,500	22,900	22,900	45,700
				Large ciliates				13,750	13,750	13,750	805,000	45,700	13,700
				Flagellates									
42	5	...do...	...do...	Small ciliates		515,000	515,000	945,000	161,000	1,560,000	13,750	13,700	13,700
				Large ciliates		6,870	6,870	6,870	6,870	6,870	41,300	13,700	6,870
				Flagellates				75,600					
43	10	...do...	...do...	Small ciliates		1,105,700	1,105,700	75,600	55,000	680,000	62,000	13,700	6,870
				Large ciliates						3,440			13,700
				Flagellates									
44	20	...do...	...do...	Small ciliates	10,320	71,200	250,000	44,700	6,870	182,000	6,870	3,435	218,000
				Large ciliates	550,000		1,375					2,740	1,370
				Flagellates								1,370	
45	50	...do...	...do...	Small ciliates	1,375	1,375	4,130		4,130	1,375	4,130		2,740
				Large ciliates	17,200	20,620	687	687		4,130	687		
				Flagellates						687			
46	100	...do...	...do...	Small ciliates	687	687	2,600	687		1,375	687		1,370
				Large ciliates	50,300	38,600							
				Flagellates									

TABLE VII.—*Comparison of the numbers of small ciliates, large ciliates, and flagellates developed daily in different artificial culture solutions from varying amounts of moist and dry soil for a period of 30 days. These numbers are calculated to 1 gm. of dry soil—Continued*

Solution No.	Quantity of soil. (Gm.)	Kind of soil.	Medium.	Kind of organisms.	11	12	13	14	15	16	17	18	19	20
10	1	Moist	Blood extract.	Small ciliates			82,500	82,500	82,500			82,500	82,500	
				Large ciliates										
				Flagellates		82,500		82,500		82,500	82,500	82,500	82,500	163,000
11	3	...do...	...do...	Small ciliates					26,800		26,800		26,800	
				Large ciliates										
				Flagellates	824,000	218,000	53,600	164,000	247,500	257,000		181,000		53,600
12	5	...do...	...do...	Small ciliates					16,500					
				Large ciliates										
				Flagellates	66,000	147,000	330,000	299,000	330,000	165,000	66,000		66,000	692,000
13	10	...do...	...do...	Small ciliates	8,250	8,250			8,250	8,250		8,250		
				Large ciliates	4,120	4,120		4,120			4,120	4,120	4,120	
				Flagellates			385,000	16,600	16,600	66,000	41,200	49,500	24,700	107,000
14	20	...do...	...do...	Small ciliates										
				Large ciliates					1,660					1,660
				Flagellates	24,700	28,800	28,800	45,400	57,700	24,700		16,500	12,400	8,250
15	50	...do...	...do...	Small ciliates	8,250									
				Large ciliates			1,660	825		1,660		1,660	825	
				Flagellates		13,200	13,200	3,300	4,950	3,300		4,950	6,600	6,600
16	100	...do...	...do...	Small ciliates										
				Large ciliates			825	825				825		
				Flagellates	5,760	2,470	4,950		1,650	2,470		3,300	1,650	4,120
20	1	Dry	...do...	Small ciliates	61,700	61,700			61,700	61,700	61,700	61,700		61,700
				Large ciliates										
				Flagellates			123,500			123,500		477,000	26,000	
21	3	...do...	...do...	Small ciliates						41,200	61,700		41,200	61,700
				Large ciliates										
				Flagellates	26,000	144,000	166,000		166,000			49,500		
22	5	...do...	...do...	Small ciliates	6,180				6,180		41,200	6,180		82,500
				Large ciliates										
				Flagellates		37,000	49,500		144,000	12,400	37,000		24,700	12,400
23	10	...do...	...do...	Small ciliates	12,360	12,360	12,360					12,360	12,360	12,400
				Large ciliates										
				Flagellates					10,300				6,180	37,000
24	20	...do...	...do...	Small ciliates			6,180	6,180	6,180	3,090	9,270	6,180	6,180	6,180
				Large ciliates										
				Flagellates	3,090	6,180	6,180	6,180	6,180	3,090	9,270	3,090	3,090	3,090

No.		%		Organism	1	2	3	4	5	6	7	8	9	10
25	do.	50	do.	Small ciliates	......	......	......	......	......	......	......	6,180	1,240	3,700
				Large ciliates	......	......	......	......	2,480	......	......	......	1,240	......
				Flagellates	618	1,240	1,230	618	618	......	2,480	3,090	2,480	......
26	do.	100	do.	Small ciliates	618	......	......	618	618	2,480	618	618	618	618
				Large ciliates	......	......	618	618	618	618	618	618	618	618
				Flagellates	......	......	......	......	......	......	......	......	......	......
30	Moist	1	Soil extract	Small ciliates	......	53,700,000	46,700,000	25,000,000	19,600,000	11,625,000	183,000	183,000	1,190,000	91,500
				Large ciliates	68,500	29,800	......	......	......	......	91,500	640,000	1,190,000	91,500
				Flagellates	137,500	......	......	......	......	......	6,470,000	29,800	29,800	825,000
31	do.	3	do.	Small ciliates	149,000	59,500	59,500	59,500	29,800	470,000	29,800	119,000	59,500	29,800
				Large ciliates	36,700	36,700	36,700	18,300	......	18,300	29,800	36,700	18,300	......
				Flagellates	18,300	18,300	18,300	18,300	18,300	18,300	179,000	......	......	29,800
32	do.	5	do.	Small ciliates	36,700	9,150	9,150	18,350	36,700	18,300	18,300	36,800	18,300	18,300
				Large ciliates	36,700	9,150	9,150	18,350	18,350	18,300	9,150	......	9,150	18,300
				Flagellates	......	91,500	91,500	137,000	146,500	9,150	9,150	36,800	9,150	9,150
33	do.	10	do.	Small ciliates	82,500	4,570	4,570	......	......	45,700	9,150	18,350	27,500	27,500
				Large ciliates	4,570	......	4,570	4,570	9,150	......	18,350	......	4,570	4,570
				Flagellates	......	9,150	4,570	16,000	10,320	13,700	9,150	59,500	105,530	13,700
34	do.	20	do.	Small ciliates	9,150	3,670	12,600	......	......	......	1,835	1,835	1,835	5,500
				Large ciliates	......	918	......	......	918	918	5,500	7,300	3,660	2,750
				Flagellates	3,670	4,590	8,250	2,750	9,180	9,180	1,835	918	3,660	918
35	do.	50	do.	Small ciliates	918	8,250	......	8,250	9,180	918	7,300	918	2,750	1,830
36	do.	100	do.	Small ciliates	4,590	......	......	......	......	......	8,250	......	......	......
40	Dry	1	do.	Small ciliates	68,500	68,500	68,500	68,500	68,500	68,500	68,500	68,500	68,500	68,500
				Large ciliates	137,500	890,500	......	......	......	......	68,500	......	68,500	68,500
				Flagellates	22,900	......	5,270,000	12,300,000	29,100,000	12,000,000	10,140,000	206,000	68,500	......
41	do.	3	do.	Small ciliates	22,900	367,000	480,000	92,000	92,000	72,000	629,000	45,700	45,700	22,900
				Flagellates	......	......	......	......	......	......	......	22,900	22,900	......
42	do.	5	do.	Small ciliates	......	13,700	......	......	69,500	......	......	......	92,000	92,000
				Large ciliates	54,800	413,000	149,000	82,500	13,700	298,000	235,000	68,700	27,400	27,400
				Flagellates	6,870	6,870	6,870	13,700	41,300	6,870	......	6,870	6,870	6,870
43	do.	10	do.	Small ciliates	27,400	......	13,700	13,700	6,870	20,600	13,700	6,870	13,700	27,400
				Large ciliates	13,700	13,700	3,440	3,440	3,440	3,440	3,440	6,950	3,440	3,440
44	do.	20	do.	Small ciliates	39,000	61,700	20,600	......	3,440	3,440	10,330	13,700	6,950	......
				Large ciliates	4,130	3,440	1,370	1,370	13,700	13,700	1,370	......	2,740	2,740
45	do.	50	do.	Small ciliates	......	34,400	......	1,370	1,370	1,370	......	......	......	......
				Large ciliates	2,740	10,940	9,620	68,700	9,130	......	1,370	6,950	2,740	687
46	do.	100	do.	Small ciliates	687	687	687	687	687	687	1,370	687	687	687
				Flagellates	......	1,370	1,370	687	687	2,060	1,370	1,370	687	2,060

TABLE VII.—*Comparison of the numbers of small ciliates, large ciliates, and flagellates developed daily in different artificial culture solutions from varying amounts of moist and dry soil for a period of 30 days. These numbers are calculated to 1 gm. of dry soil*—Continued

Solution No.	Quantity of soil	Kind of soil	Medium	Kind of organisms	Days after inoculation									
					21	22	23	24	25	26	27	28	29	30
	Gm.													
10	1	Moist	Blood extract	Small ciliates	82,500			82,500	165,000		330,000	165,000	247,000	165,000
				Large ciliates	82,500					82,500	82,500	82,500	165,000	
				Flagellates	165,000	165,000	165,000	165,000	330,000	26,800	165,000	26,800	165,000	26,800
11	3	do	do	Small ciliates	26,800	80,400	53,000	26,800	26,800	16,000	53,600	80,400	80,400	640,000
				Large ciliates		99,000	16,600	16,600		16,000	33,200	99,000	16,600	16,600
				Flagellates		16,600								
12	5	do	do	Small ciliates	99,000	16,600	99,000	115,000	181,000	82,500	165,000	82,500	99,000	198,000
				Large ciliates	8,250	33,200		8,250		8,250	33,200	8,250		
				Flagellates										
13	10	do	do	Small ciliates	24,700	107,000	16,600	82,500	90,700	66,000	82,500	41,250	49,500	99,000
				Large ciliates		8,250	4,125	4,125	8,250		4,125		4,125	
				Flagellates										
14	20	do	do	Small ciliates	116,400	107,000	16,500	20,600	4,125	8,250	16,500	4,125	33,000	4,125
				Large ciliates		4,940		1,650		1,650	1,650	1,650	4,940	53,600
				Flagellates				3,300	1,650					1,650
15	50	do	do	Small ciliates	1,650	1,650		1,650	1,650	1,650	1,650	1,650		1,650
				Large ciliates	1,650	1,650		825			825			
				Flagellates	1,650		1,650	825			1,650		825	1,650
16	100	do	do	Small ciliates		825		825	1,650	825		825	825	825
				Large ciliates	1,650	4,125	1,650	5,775	1,650		1,650			1,650
				Flagellates			5,775	5,775						
20	1	Dry	do	Small ciliates			61,800	123,800	61,800	61,800	550,000	495,000	1,122,000	3,800,000
				Large ciliates		61,800	61,800	61,800		123,800				
				Flagellates			61,800	61,800		20,600				
21	3	do	do	Small ciliates	20,600	206,000	61,800	144,000	144,000	61,800	41,200	103,000	123,500	144,000
				Large ciliates		12,350		12,350			12,350			
				Flagellates										
22	5	do	do	Small ciliates	12,350	37,050		24,700	12,350	49,500	174,000	149,000	262,000	257,000
				Large ciliates									6,180	
				Flagellates										
23	10	do	do	Small ciliates	6,180	18,540	12,360	24,700	12,360	18,540	12,360	6,180	12,360	12,360
				Large ciliates	6,180	6,180	6,180	3,090	6,180	3,090	9,270	3,090	3,090	
				Flagellates	3,090				3,090					
24	20	do	do	Small ciliates	6,180	12,360	6,180	9,270	6,180		6,180	3,090	3,090	6,180
				Flagellates										

25	50	...do....	.....do........	Small ciliates....	3,700	3,700	3,700	1,240	1,240		1,240	1,240		
				Large ciliates....						1,240				
				Flagellates......				1,240			1,240		1,240	
26	100	...do....	.....do........	Small ciliates....				1,230	2,460	3,690	618	618	618	618
				Large ciliates....		618	618	618				618	618	
				Flagellates......	1,230	1,230	618	618	1,230	1,850	3,090	2,470	1,850	4,950
30	1	Moist...	Soil extract...	Small ciliates....	92,000	183,000	183,000	183,000	92,000	92,000	275,000	275,000	183,000	92,000
				Large ciliates....	92,000	92,000				92,000	92,000		92,000	
				Flagellates......	183,000	92,000								
31	3	...do....	.....do........	Small ciliates....	29,800		89,300	29,800						
				Large ciliates....			59,600					29,800		
				Flagellates......	29,800	59,600			59,600	29,800		29,800		
32	5	...do....	.....do........	Small ciliates....			4,570				36,600	55,000	36,600	
				Large ciliates....										
				Flagellates......	18,300	6,400	13,700	36,600	36,600	18,300		73,500	36,600	55,000
33	10	...do....	.....do........	Small ciliates....	9,200		18,300	9,200	9,200		18,300	9,200	9,200	9,200
				Large ciliates....									9,200	
				Flagellates......	9,200		18,300	18,300	18,300	9,200	9,200	18,300	9,200	18,300
34	20	...do....	.....do........	Small ciliates....			9,200	4,570	4,570	13,700	4,570			
				Large ciliates....										
				Flagellates......	9,200	18,300		13,700	9,200	9,200	13,700	32,000	18,320	9,200
35	50	...do....	.....do........	Small ciliates....	1,830		1,830	1,830	1,830			1,830		
				Large ciliates....										
				Flagellates......	3,670	13,700	1,370	49,300	63,000	28,700	18,350	50,500	27,250	88,300
36	100	...do....	.....do........	Small ciliates....	2,740	2,740	1,830	1,830	2,740	918	1,830	1,830	1,830	2,740
				Large ciliates....		918			918		918			
				Flagellates......	918	1,830		918	918			2,740	918	
40	1	Dry.....	.....do........	Small ciliates....	137,500	137,500	68,700	68,700	137,500		68,700			
				Large ciliates....	68,700	68,700	68,700	68,700	68,700	68,700	68,700	68,700	68,700	68.700
				Flagellates......		68,700	620,000	137,500	137,500	137,500	68,700	275,000	68,700	413,000
41	3	...do....	.....do........	Small ciliates....	22,900				22,900					22,900
				Large ciliates....			22,900							22,900
				Flagellates......	22,900	45,800	68,700	68,700	45,800		22,900	22,900		
42	5	...do....	.....do........	Small ciliates....		13,700	13,700							
				Large ciliates....		13,700								
				Flagellates......	13,700	27,400	96,500	27,400	27,400	41,300	13,700	27,400	13,700	27,400
43	10	...do....	.....do........	Small ciliates....	6,870	1,370	6,870	20,600	6,870	6,870	6,870	6,870	6,870	
				Large ciliates....				6,870			6,870			
				Flagellates......	1,370	34,400	1,370	6,870	13,700	20,600	13,700	13,700		
44	20	...do....	.....do........	Small ciliates....	3,440		6,870	3,440	3,440		3,440	3,440		
				Large ciliates....										
				Flagellates......	3,440	6,870	6,870	3,440			3,440	3,440	3,440	3,440
45	50	...do....	.....do........	Small ciliates....	1,370	2,740	2,740	2,740	4,130	1,370		2,740	1,370	1,370
				Large ciliates....		1,370								
				Flagellates......	1,370	2,740	1,370	1,370	2,740	1,370	1,370	1,370	1,370	12,400
46	100	...do....	.....do........	Small ciliates....	687	1,370	2,750	687	1,370		2,060	687	687	
				Large ciliates....					687					
				Flagellates......	687	5,500	4,800	4,130	6,170	2,060	1,370	2,750	687	4,130

LARGE CILIATES.—Very few large ciliates developed in any of the inoculated solutions. These organisms appeared on the first day in some moist soil inoculations of dried-blood extract. In blood extract to which dry soil had been added and soil extract inoculated with moist soil large ciliates appeared on the second day in some solutions and on the third day in soil extract containing the largest amounts of dry soil. In all inoculations of dried-blood extract the greatest numbers appeared from the third to the eighth day; then the numbers decreased, so that there were very few in any of the solutions after the ninth day. In soil extract the organisms appeared much sooner in solutions with the larger than in the solutions with the smaller soil inoculations. As noted in Table VII, in solution 36, with 100 gm. of moist soil, the organisms developed on the second day, while in solution 30, when 1 gm. was used, the organisms did not appear until the nineteenth day. As many large ciliates developed in the dried blood as in soil extract. Again there was no definite relation between the numbers of organisms developed and the quantities of soil used for inoculation.

FLAGELLATES.—As noted in Table VII, in all inoculations of dried-blood extract, the maximum number of flagellates were present at an earlier period than in inoculations of soil extract. The maximum development in dried blood was from the second to the fourth day, depending on the quantity and kind of soil used. The largest number of organisms appeared sooner in solutions inoculated with the largest amounts of soil than where small quantities were used. With 100-gm. inoculations the maximum development of flagellates in solutions of soil extract with both moist and dry soils was on the seventh day, while with 1 gm. of moist soil the greatest numbers of organisms appeared on the eleventh day, and on the fifteenth day with the same amount of dry soil. As soon as the maximum numbers were reached, there was a gradual decrease until but few organisms remained in the solutions. In culture solutions inoculated with moist and dry soils, the largest development of flagellates was reached in the soil extract with the smallest quantities of soil, while with the largest amounts of soil the greatest development was in dried blood. In soil extract, with one exception, a larger number of flagellates were developed from the dry than from moist soil; this, however, was not the case with inoculations in blood. In nearly all inoculations with dry soil, the greatest numbers were developed in soil extract. In all cases there were nearly 200 times as many organisms developed per gram, from the 1-gm. than from the 100-gm. inoculations. In inoculated solutions of blood extract, the maximum number of all organisms appeared between the third and fourth day, while in the soil extract the largest number were present from the second to the fifteenth day, depending upon the kind and amount of soil inoculated.

DEVELOPMENT OF PROTOZOA FROM DIFFERENT COMPOST SOILS

As shown in Table VIII, the development of small ciliates, with respect to amounts of soil and media, was practically identical with the development in moist soil, as shown in Table VII. It is apparent from Table VIII that more small and more large ciliates develop from the less composted soils. In all inoculations of dried blood with the 10 and 20 per cent composts and for the larger amounts inoculated from the 30 and 50 per cent composts the maximum number of flagellates were present from the sixtieth hour to the fourth day. But with smaller amounts of soil inoculations from the 30 and 50 per cent manure soils, the maximum numbers appeared from the fourth to the seventh day. In the case of dried blood there was little difference in the numbers of flagellates developed from the different amounts of soil, while in soil extract inoculated with the smallest quantities of soil the maximum had not yet been reached at the end of the eighth day; hence, no comparison could be made. From the 30 and 50 per cent composts more flagellates were developed in blood extract than from the 10 and 20 per cent manures. Considering the numbers of organisms developed, on the gram basis, with dried blood there were more than two hundred times as many flagellates developed from 1 gm. as compared with 100 gm. of soil. It is seen that the maximum number of all organisms developed from the sixtieth hour to seven days in dried blood, while in the case of soil extract the maximum numbers had not been reached for the smaller soil inoculation when the experiment was concluded.

DEVELOPMENT OF DIFFERENT TYPES OF PROTOZOA FROM THE SOIL

Very many different types of large ciliates were developed. Of the forms identified *Paramecium* sp. was probably the most common. Other large ciliates which were noted were probably *Encheyls pupa* Ehrb., a few individuals of *Urolaptus musculus* Ehrb., and probably *Nassula elegans* Ehrb. The vorticellæ were very numerous, appearing the fourth day, and were present in some solutions throughout the period of 30 days. They were developed in dried blood and soil extract from both moist and dry soils. Next in prominence was the *Colpoda cucullus* O. F. M., which was first recognized on the fourth day. *Colpidium colpoda* Ehrb. was also quite common. Of the flagellates species resembling *Monas guttula* Ehrb. and *Monas vivipara* Ehrb. were the most common. *Bodos* spp. were also very common. A few dimastigate amœbæ, apparently *Amoeba radiata* Kelbs, were seen between the fifteenth and twentieth day after inoculation. A species corresponding to *Peranema trichophorum* Ehrb. appeared in small numbers from the eighteenth to the twenty-second day. Likewise a few organisms resembling *Trinema anchelys* Ehrb. were observed. Very few amœbæ

TABLE VIII.—*Comparison of the numbers of small ciliates, large ciliates, and flagellates developed daily in different artificial culture solutions and from varying amounts of different greenhouse soils for a period of 8 days. These numbers are all calculated to 1 gm. of dry soil*

Solution No.	Quantity of soil	Kind of soil	Medium	Kind of organisms	12 hours	36 hours	60 hours	4th day	5th day	6th day	7th day	8th day
	Gm.											
201	1	10 per cent compost	Soil extract	Small ciliates						87,300		
				Large ciliates						87,300		
				Flagellates								
202	20	......do......	......do......	Small ciliates			4,350	172,000	4,350	4,350	4,350	4,350
				Large ciliates				22,300				
				Flagellates								
203	50	......do......	......do......	Small ciliates			42,300	91,500	56,600	87,000	5,160	1,730
				Large ciliates			1,720	3,440	1,720	3,440	4,350	3,440
				Flagellates			4,350			870	870	870
204	100	......do......	......do......	Small ciliates		7,900	84,600	613,000	50,500	1,720	870	4,350
				Large ciliates				221,600	870	1,720		
				Flagellates				7,180	1,720			
211	1	......do......	Blood extract	Small ciliates			78,300	1,590,000	78,300	79,300	7,830	79,300
				Large ciliates				78,300	158,800			
				Flagellates		235,000	8,520,000	32,200,000	2,680,000	2,180,000	1,340,000	2,180,000
212	20	......do......	......do......	Small ciliates	3,920	15,650	37,000	58,700	7,850	15,625	1,550	
				Large ciliates								
				Flagellates			1,550	1,550	1,550	1,550	1,550	
213	50	......do......	......do......	Small ciliates	1,540	3,045	288,000	212,000	12,350	9,370	3,990	3,090
				Large ciliates								
				Flagellates			785			785	785	1,550
214	100	......do......	......do......	Small ciliates	3,045	7,820	168,000	52,500	5,150		785	3,990
				Large ciliates								
				Flagellates								1,550
101	1	20 per cent compost	Soil extract	Small ciliates					367,000	91,500	91,500	91,500
				Large ciliates								
				Flagellates								
102	20	......do......	......do......	Small ciliates				745,000	660,000	497,000	27,500	55,000
				Large ciliates			4,570	4,570		4,570	4,570	4,570
				Flagellates			9,150	3,670			3,670	
103	50	......do......	......do......	Small ciliates			3,859,000	6,730,000	62,000	33,200	9,150	18,300
				Large ciliates			697			697	697	
				Flagellates	1,830	1,830		1,100				4,130
104	100	......do......	......do......	Small ciliates		3,440	149,700	172,000	2,750	9,650	7,550	25,200
				Large ciliates								
				Flagellates								

No.	Compost	Dilution	Extract	Organism								
111		1	Blood extract	Small ciliates	....	....	....	82,500	165,000	165,000	359,000	82,500
				Large ciliates	....	82,500	82,500	....	82,500	82,500	247,000	82,500
				Flagellates	3,910,000	49,800,000	80,600,000	660,000	....	....	4,120	165,000
112	....do.	20	....do.	Small ciliates	241,000	162,000	61,800	8,250	28,800	24,700	4,120	165,000
				Large ciliates	1,650	3,300	9,880	825	1,650	....	....	3,300
				Flagellates	63,800	230,500	3,300	825	1,650	....	1,650	19,800
113	....do.	50	....do.	Small ciliates	1,650	825	171,000	24,700	....	6,000	13,200	....
				Large ciliates	....	825	3,300	....	....	....	....	....
				Flagellates	....	....	52,500	4,950	800	....	4,120	19,800
114	....do.	100	....do.	Small ciliates	8,240	....	....	4,950	1,650	....	4,120	8,250
				Large ciliates	49,500	....	80,300	....	....	80,300	....	80,300
				Flagellates	....	....	....	....	....	....	....	....
401	30 per cent compost	1	Soil extract	Small ciliates	....	....	80,300	4,020	12,000	4,020	....	8,000
				Large ciliates	....	....	....	....	....	....	....	....
402	....do.	20	....do.	Small ciliates	4,020	64,700	1,650,000	408,000	16,000	143,000	261,000	
				Large ciliates	1,600	1,600	4,820	3,210	....	1,600	1,600	
403	....do.	50	....do.	Small ciliates	1,600	440,000	43,500	48,200	3,200	17,600	12,700	
				Large ciliates	11,170	....	....	1,600	800	....	1,600	
				Flagellates	800	....	....	....	....	....	....	
404	....do.	100	....do.	Small ciliates	47,700	147,500	87,500	86,500	800	2,400	4,020	
				Large ciliates	4,020	....	....	....	....	....	....	
411		1	Blood extract	Small ciliates	....	21,400,000	24,900,000	23,500,000	1,915,000	144,000	1,019,000	
				Large ciliates	217,000	....	....	....	3,600	3,600	3,600	
412	....do.	20	....do.	Small ciliates	3,600	137,000	172,000	171,000	129,000	7,200	32,400	
				Large ciliates	173,000	1,440	4,330	2,890	....	1,440	1,440	
413	....do.	50	....do.	Small ciliates	1,440	379,000	99,900	99,390	2,890	1,440	2,880	
				Large ciliates	85,000	....	....	1,440	720	....	1,440	
414	....do.	100	....do.	Small ciliates	4,330	41,800	7,200	7,200	1,440	720	1,440	
				Large ciliates	720	....	....	....	....	....	....	
				Flagellates	9,370	....	98,500	98,500	....	....	....	
301	50 per cent compost	1	Soil extract	Small ciliates	2,880	192,000	....	3,120,000	690,000	....	1,019,000	
				Large ciliates	....	....	4,930	4,930	29,600	4,930	3,600	
302	....do.	20	....do.	Small ciliates	....	....	....	....	....	....	4,930	
303	....do.	50	....do.	Small ciliates	....	....	305,000	590,000	51,500	33,200	55,700	
				Large ciliates	985	93,500	....	....	....	....	....	
304	....do.	100	....do.	Small ciliates	985	233,000	202,000	4,930	19,800	38,800		

TABLE VIII.—*Comparison of the numbers of small ciliates, large ciliates, and flagellates developed daily in different artificial culture solutions and from varying amounts of different greenhouse soils for a period of 8 days. These numbers are all calculated to 1 gm. of dry soil*—Continued

Solution No.	Quantity of soil	Kind of soil	Medium	Kind of organisms	Period after inoculation							
					12 hours.	36 hours.	60 hours.	4th day.	5th day.	6th day.	7th day.	8th day.
	Gm.											
311	1	50 per cent compost	Blood extract	Small ciliates								
				Large ciliates		88,500						
				Flagellates		266,000	14,200,000	3,660,000	532,000	29,400,000	44,000,000	26,270,000
312	20	do	do	Small ciliates		4,420	4,420					4,430
				Large ciliates								
				Flagellates		177,700	149,500	1,570,000	87,500	39,200	22,200	26,600
313	50	do	do	Small ciliates		7,120	422,000	299,000	37,100	3,500	5,250	8,850
				Large ciliates								
				Flagellates								
314	100	do	do	Small ciliates		835	17,800	241,000	16,800			885
				Large ciliates								
				Flagellates								

were developed; one large form was developed from moist soil in dried-blood extract on the eighth day; thereafter no forms were recognized until the twenty-third and twenty-fourth day, when a few small forms were noted. Protozoan cysts were very numerous in the former part of the experiment, but they gradually disappeared until very few were seen after the twenty-second day, thus indicating that in culture solutions some of the protozoa do not encyst after they have once become active, but either die or are destroyed by other forms.

SUMMARY OF PART II

Under the conditions of the experiment and with the soils examined it was found that:

(1) In culture solutions the maximum development of small and large ciliates and flagellates varies with the culture solution and the condition and amounts of soil used for inoculation.

(2) In dried-blood extract the maximum development of all ciliates and flagellates is from the third to the fourth day, while in soil extract it is from the second to the fifteenth day, depending upon the character and amount of soil used for inoculation.

(3) When the maximum development of all organisms is reached, there is a gradual decrease in numbers until very few active forms are present.

(4) The greatest numbers of protozoa developed sooner in culture solutions inoculated with the largest quantities of soil.

(5) Per gram of soil, there is the greatest development from the least amount of soil used for inoculations.

(6) For the development of all forms soil extract seemed to be a little more favorable than dried-blood extract.

(7) The flagellates are the first organisms to excyst.

(8) Very few large and small ciliates developed as compared with the numbers of flagellates.

(9) Drying the soil slightly favored the development of flagellates in soil extract, while with dried blood there was little difference.

(10) More large and small ciliates developed from the less composted soils.

(11) In dried blood more flagellates developed from the more heavily manured soils.

(12) Very many different types of ciliates were present, while the types and numbers of amœbæ were few.

III.—PROTOZOA OF FIELD AND GREENHOUSE SOILS

INTRODUCTION

The work reported in Part II of this paper on the protozoa of greenhouse soils led the writer to make a more complete investigation of the development of these organisms in culture solutions. In earlier experi-

ments it was found that the development of the different types of protozoa varied greatly with the culture solutions employed in studying these organisms.

The purpose of this problem was to study:

(1) The development of protozoa in different culture solutions.

(2) The development of protozoa from varying amounts of soil inoculations.

(3) The comparison of the numbers and types of protozoa developed from compost and field soils.

A large sample of the same 20 per cent compost greenhouse soil containing 24.30 per cent of moisture which was used in the work on greenhouse soils in Part II was collected from a bin in the greenhouse. Likewise a sample of a heavy clay field soil which had not received fertilizer for several years was collected from a young orchard. This soil, which was taken 3 inches from the surface, had a moisture content of 18.35 per cent, and the temperature at the time of collection was 0.5° C. To 100 c. c. portions of 3 per cent dried-blood extract with 0.05 per cent of dibasic potassium phosphate and the same amount of a 10 per cent hay infusion [1] were inoculated with 1, 5, 20, 50, and 100 gm. portions of each soil. The inoculated solutions were examined for protozoa and then incubated at 22° C. for a period of 30 days. At the same hour each day counts were made of the organisms developed. The examinations were made under the low power of the microscope and the organisms enumerated by the improved loop method as described in Part I of this paper. When the specially prepared slide was not used, in counting culture solutions containing more than 350,000 organisms per cubic centimeter the loop of culture solution was transferred to a plain glass slide, a 5 mm. square of which was carefully ruled off into 40 to 50 small fields of equal size. All the organisms in the incomplete fields on the outer portions of the film of culture solution were counted, the average of the numbers in several fields were taken, and the numbers of organisms calculated as in the improved loop method. This method checked very closely with counts made by means of the special slide.

As in the previous work, the same difficulty of distinguishing large bacteria from small flagellates was encountered. Hence, to facilitate the enumeration of the organisms it was thought advisable to make all of the counts under the low power of the microscope. In many cases it was difficult to distinguish small ciliates from flagellates; hence, notwithstanding precautions taken in enumerating the organisms, it is quite probable that some small ciliates might have been counted as flagellates, and vice versa.

[1] This formula was recommended by N. Kopeloff, H. Clay Lint, and David A. Coleman in 1915, in an unpublished manuscript entitled, "A New Method for the Counting of Protozoa and Some Media for Their Development."

In this study the same classification of protozoa was made as in previous experiments. The small ciliates included all organisms from the smaller to and including *Colpidium colpoda*. The vorticellæ were also included. The large ciliates included all forms larger than *Colpidium colpoda*. The flagellates included all forms of flagellates that were observed.

DEVELOPMENT OF PROTOZOA IN CULTURE SOLUTIONS INOCULATED WITH VARIOUS AMOUNTS OF SOIL

DEVELOPMENT OF SMALL CILIATES

From Table IX it is apparent that the development of small ciliates varies greatly with the kind of media and soil used. It is noted that but few of these organisms developed in any of the solutions of dried-blood extract inoculated with the varying quantities of field soil. Very many appeared from the 5-gm. and 20-gm. inoculations of the same soil in hay infusion, indicating that only definite types develop under certain conditions. That this is true is again apparent. In hay infusion inoculated with 100 gm. of field soil only a few organisms developed on the second and third days. In the 1-gm. and 50-gm. inoculations no small ciliates had developed during the whole period of 30 days, but in the case where 100 c. c. of hay infusion had been inoculated with 5 gm. of soil, as many as 30,700,000 organisms per gram were present on the eighth day. With the 20-gm. inoculations 3,700,000 had appeared on the ninth day. In both media more small ciliates developed from the compost than from the field soil. In the compost inoculations of both dried blood and hay infusion a few organisms appeared on the second day where the largest quantities of soil had been used. But the maximum numbers were not reached until the fourth day with the former and the fifth day with the latter solution. In the development of the maximum numbers of small ciliates in the different culture solutions, a variation in the period of the development was very apparent. In all inoculations of dried blood the greatest numbers were noted on the third and fourth days. With hay infusion the maximum numbers did not appear until the fifth, and in some cases not until the eighth day, depending upon the amount of soil used for inoculation.

DEVELOPMENT OF LARGE CILIATES

No large ciliates were noted in any of the inoculations of the field soil, indicating that either the conditions in the media must have been unfavorable for their development or that there were no cysts of large ciliates in the soil.

That such cysts are present in greenhouse soil is indicated by the development of these organisms in both dried blood and hay infusion. The former solution, however, did not seem to be very favorable for their

TABLE IX.—*Comparison of the numbers of large and small ciliates and flagellates which developed daily in different artificial culture solutions inoculated with varying quantities of field and greenhouse soils for a period of 30 days. These numbers have all been calculated on the basis of 1 gm. of dry soil*

Solution No.	Quantity of soil (Gm.)	Kind of soil	Medium	Kind of organisms	Days after inoculation									
					1	2	3	4	5	6	7	8	9	10
801	1	Field	Blood extract	Small ciliates					63,000	125,500	125,500	188,500	63,000	63,000
				Large ciliates		151,500	10,052,000	9,700,000	2,020,000	125,500	125,500	627,000	1,079,000	525,000
				Flagellates										
802	5	...do...	...do...	Small ciliates	14,300	515,000	5,200,000	14,000,000	13,200,000	6,400,000	3,020,000	835,000	1,245,000	1,009,000
				Large ciliates			3,610		3,090					3,210
				Flagellates										
803	20	...do...	...do...	Small ciliates		15,150	2,100,000	1,400,000	949,000	707,000	290,000	323,000	72,600	21,600
				Large ciliates						1,235	1,235	1,235	1,285	1,285
				Flagellates										
804	50	...do...	...do...	Small ciliates		2,990	202,000	381,000	212,000	78,300	113,000			
				Large ciliates								2,470	2,570	1,285
				Flagellates										
805	100	...do...	...do...	Small ciliates		5,250	151,500	172,000	62,300	351,000	234,000	113,000	71,700	
				Large ciliates										1,285
				Flagellates										
811	1	Greenhouse	...do...	Small ciliates				245,000	68,000		204,000	136,000	136,000	136,000
				Large ciliates		1,215,000	29,200,000	72,200,000	612,000	544,000	272,000	136,000	475,000	493,000
				Flagellates			65,000	1,515,000	13,400	13,400				
812	5	...do...	...do...	Small ciliates	15,450	342,000	3,930,000	130,000	26,800	94,700	40,200	26,800	216,000	1,330,000
				Large ciliates			36,800	12,400			3,400			3,400
				Flagellates										
813	20	...do...	...do...	Small ciliates			98,500	131,000			3,400	3,400		
				Large ciliates							4,120	1,340	1,340	1,340
				Flagellates	8,150	24,500	630,000	122,500	54,700	6,800	44,300	10,200	10,200	6,800
814	50	...do...	...do...	Small ciliates	30,300	311,000	33,000	117,400						1,340
				Large ciliates							5,460	5,460	5,450	1,340
				Flagellates			2,360	3,200	2,680	1,340				620
815	100	...do...	...do...	Small ciliates										
				Large ciliates										
				Flagellates	42,200	474,000	19,550	6,500			1,960			1,340
821	1	Field	Hay infusion	Small ciliates		314,000	236,000	16,950,000	33,600,000	62,600,000	202,000,000	27,800,000	39,400,000	40,200,000
				Large ciliates						117,500	1,825,000	30,700,000	6,950,000	1,970,000
				Flagellates										
822	5	...do...	...do...	Small ciliates			236,000	6,700,000	8,950,000	7,300,000	50,800,000	10,700,000	11,900,000	209,800
				Large ciliates										
				Flagellates										

823	20	do	do	Small ciliates	....	3,960	....	....	6,540	65,400	60,000	3,260,000	3,730,000	400,000
				Large ciliates	....	....	....	....	....	....	....	....	....	....
				Flagellates	....	19,250	240,000	3,360,000	6,220,000	12,300,000	5,400,000	12,850	....	....
824	50	do	do	Small ciliates	....	....	....	....	....	....	....	....	....	....
				Large ciliates	....	....	....	....	....	....	....	....	....	....
				Flagellates	....	9,420	81,500	500,000	23,600	287,000	882,000	2,310,000	500,000	344,000
825	100	do	do	Small ciliates	....	750	750	....	....	....	....	....	....	....
				Large ciliates	....	....	....	....	....	....	....	....	....	....
				Flagellates	....	....	3,100	130,500	3,960	20,900	24,600	1,210,000	1,122,000	78,200
831	1	Greenhouse	do	Small ciliates	....	....	....	141,000	17,100,000	177,500,000	102,720,000	25,550,000	3,700,000	24,800,000
				Large ciliates	....	....	....	....	....	....	....	....	....	....
				Flagellates	84,500	32,900,000	507,000	73,100,000	211,300,000	....	57,250,000	13,570,000	3,110,000	5,380,000
832	5	do	do	Small ciliates	....	....	....	....	....	....	....	....	....	....
				Large ciliates	....	....	....	....	197,000	365,000	....	197,000	395,000	940,000
				Flagellates	16,050	1,165,000	11,380,000	5,150,000	1,350,000	27,800	15,000,000	9,570,000	75,800,000	70,500,000
833	20	do	do	Small ciliates	....	....	140,000	545,000	665,000	1,120,000	1,080,000	665,000	765,000	417,000
				Large ciliates	....	....	7,500	7,050	42,800	63,200	....	13,900	....	7,050
				Flagellates	4,280	21,400	2,500,000	1,430,000	1,070,000	....	....	21,400	7,070	13,900
834	50	do	do	Small ciliates	....	1,715	139,000	471,000	400,000	535,000	385,000	416,000	146,600	101,600
				Large ciliates	....	....	....	7,050	27,800	41,700	11,780	11,780	....	....
				Flagellates	1,715	205,300	278,000	750,000	141,000	....	....	5,670	5,670	22,450
835	100	do	do	Small ciliates	....	2,460	43,800	88,200	94,700	39,600	26,750	26,750	33,100	13,900
				Large ciliates	....	....	....	....	5,670	4,170	....	....	....	1,390
				Flagellates	750	21,900	175,500	4,930	7,130	....	8,450	4,170	2,130	25,500

TABLE IX.—*Comparison of the numbers of large and small ciliates and flagellates which developed daily in different artificial culture solutions inoculated with varying quantities of field and greenhouse soils for a period of 30 days. These numbers have all been calculated on the basis of 1 gm. of dry soil*—Continued

Solution No.	Quantity of soil (Gm.)	Kind of soil	Medium	Kind of organisms	Days after inoculation									
					11	12	13	14	15	16	17	18	19	20
801	1	Field	Blood extract	Small ciliates										
				Large ciliates	371,000	125,500	251,000	125,500	567,000	315,000	126,000	380,000	63,000	251,000
				Flagellates					12,350	12,350	12,350	12,350	12,350	24,700
802	5	....do....	....do....	Small ciliates										
				Large ciliates	480,000	63,000	75,700	12,350	24,700	63,000	226,500	9,630	226,500	9,270
				Flagellates										
803	20	....do....	....do....	Small ciliates										
				Large ciliates	3,210	3,210	6,420	6,420	6,420	9,630	9,630	9,630	3,210	15,150
				Flagellates										
804	50	....do....	....do....	Small ciliates										
				Large ciliates	1,285	1,285	3,855	1,285	16,350		2,570	3,855	1,285	2,580
				Flagellates	647									
805	100	....do....	....do....	Small ciliates										
				Large ciliates	1,285	3,855	647	647		647	1,285	647	647	
				Flagellates										
811	1	Greenhouse	....do....	Small ciliates	68,000	68,000								
				Large ciliates	68,000	68,000	68,000	136,000	136,000	1,174,000	136,000	349,000	136,000	136,000
				Flagellates									13,400	13,380
812	5	....do....	....do....	Small ciliates										
				Large ciliates	243,000	325,000	340,000	26,800	216,000	243,000	203,000	81,500	135,000	135,000
				Flagellates							3,400			
813	20	....do....	....do....	Small ciliates										
				Large ciliates	3,400		3,900	3,400	6,800	170,000	6,800		6,800	6,800
				Flagellates	1,349				2,680		1,340		1,340	
814	50	....do....	....do....	Small ciliates										
				Large ciliates	24,700	1,340	4,120	4,120	2,680	6,800	2,680	1,340	5,350	2,680
				Flagellates		1,340	620							620
815	100	....do....	....do....	Small ciliates										
				Large ciliates	620	1,340	1,340	1,340	620	1,340	620	1,965	1,340	1,340
				Flagellates										
821	1	Field	Hay infusion	Small ciliates										
				Large ciliates	76,700,000	36,400,000	26,550,000	6,150,000	1,112,000	197,000	123,000	1,505,000	123,500	246,000
				Flagellates	131,700	157,500	183,000	104,860	104,860	65,300	131,800	78,200	25,700	38,500
822	5	....do....	....do....	Small ciliates										
				Large ciliates	657,000	1,860,000	657,000	656,000						
				Flagellates					78,200					

No.	Dil.	Soil	Organism										
823	20	...do...	Small ciliates	428,000	46,000	38,500	....	32,100	3,210	3,210	9,630	3,210	12,850
			Large ciliates	38,500	25,700	6,420	12,850	3,210	....	3,210	3,210	6,420	3,210
			Flagellates	....	....	....	144,500	....	331,000	....	....	....	....
824	50	...do...	Small ciliates	668,000	530,000	130,500	....	49,700	....	184,000	226,800	151,000	2,560
			Flagellates	....	....	....	6,520	....	3,210	....	....	....	....
825	100	...do...	Small ciliates	82,500	60,000	25,700	4,800,000	6,520	2,550,000	1,925	3,090,000	642	642
			Large ciliates	10,325,000	8,770,000	10,700,000	706,000	3,600,000	70,600	9,650,000	70,500	2,420,000	1,287,000
			Flagellates	847,000	564,000	282,500	112,500	850,000	903,000	202,000	1,632,000	70,500	212,000
831	1	Greenhouse	Large ciliates	....	....	....	338,000	27,800	338,000	2,420,000	1,000,000	957,000	848,000
			Flagellates	170,500	37,000,000	8,550,000	63,000	183,000	49,200	664,000	38,500	13,900	169,000
832	5	...do...	Small ciliates	130,500,000	219,500	49,200	....	64,200	....	84,500	7,050	351,000	17,150
			Large ciliates	1,273,000	7,050	....	48,200	3,530	5,670	3,530	4,170	13,900	3,530
			Flagellates	13,900	....	39,600	....	7,060	....	....	....	3,525	4,170
833	20	...do...	Small ciliates	....	45,000	....	13,900	16,050	2,140	2,780	640	1,390	....
			Flagellates	82,500	....	....	1,390	....	....	....	....	....	642
824	50	...do...	Small ciliates	....	95,800	2,785	....	10,590	1,390	3,530	2,030	1,390	642
			Flagellates	90,800	13,900	24,600	....	....	....	....	....	2,780	2,033
835	100	...do...	Small ciliates	19,800	....	....	....	....	....	640	....	....	....
			Large ciliates	....	....	....	....	....	....	....	....	640	....
			Flagellates	2,890	4,180	....	....	....	1,390	640	....	....	....

TABLE IX.—*Comparison of the numbers of large and small ciliates and flagellates which developed daily in different artificial culture solutions inoculated with varying quantities of field and greenhouse soils for a period of 30 days. These numbers have all been calculated on the basis of 1 gm. of dry soil*—Continued

Solution No.	Quantity of soil	Kind of soil	Medium	Kind of organisms	Days after inoculation									
	Gm.				21	22	23	24	25	26	27	28	29	30
801	1	Field	Blood extract	Small ciliates										
				Large ciliates	125,500	188,500	230,700	188,500	125,500	6,450	125,500	251,000	251,000	592,000
				Flagellates		12,350								
802	5	do	do	Small ciliates										
				Large ciliates	37,100	24,700	63,000	63,000	37,100	12,900	12,900	12,900	75,200	24,700
				Flagellates								3,090		
803	20	do	do	Small ciliates										
				Large ciliates										
				Flagellates	3,090	6,180	9,270	6,180	3,090	6,180	3,090		9,270	6,180
804	50	do	do	Small ciliates										
				Large ciliates										
				Flagellates	3,710	1,290	6,180	3,710	6,180	1,290	3,710	1,290	5,160	1,290
805	100	do	do	Small ciliates										
				Large ciliates										
				Flagellates	647	647		1,290	647	647	647		1,290	
811	1	Greenhouse	do	Small ciliates										
				Large ciliates										
				Flagellates	68,000	68,000	136,000	136,000	136,000	68,000			136,000	
812	5	do	do	Small ciliates			81,500							
				Large ciliates						13,400	13,400			
				Flagellates	149,500	68,000	26,800	80,400	80,400	108,000	108,000	121,500	26,800	26,800
813	20	do	do	Small ciliates					13,500					
				Large ciliates				19,200						
				Flagellates	3,400	3,400	3,400	1,340	1,340	6,800	3,400	6,800		3,400
814	50	do	do	Small ciliates										
				Large ciliates										
				Flagellates	1,340	5,390	2,680	6,800	6,800	5,390	1,340	2,680	1,340	2,680
815	100	do	do	Small ciliates										
				Large ciliates										
				Flagellates		620	620	620		1,340			620	620
821	1	Field	Hay infusion	Small ciliates										
				Large ciliates										
				Flagellates	492,000	123,000	370,000	3,275,000	2,630,000	2,750,000	1,830,000	2,287,000	25,650,000	63,200,000
822	5	do	do	Small ciliates	38,500	51,400	25,700	38,590	12,850	12,850	25,700	12,850	12,850	12,850
				Large ciliates										
				Flagellates					25,700	25,700	12,850	38,500	12,850	12,850

No.	Depth	Location	Organism	1	2	3	4	5	6	7	8	9	10
823	20	…do…	Small ciliates	12,850	12,850	105,000	36,400	6,420	3,210	6,420	3,210	6,420	3,210
			Large ciliates						6,420		16,050	9,630	25,700
			Flagellates										
824	50	…do…	Small ciliates										
			Large ciliates	2,500	3,850	3,850	1,280	7,700	2,560	2,500	1,285	1,285	
			Flagellates										
825	100	…do…	Small ciliates										
			Large ciliates								1,285		
			Flagellates	642	642	6,530	1,920	1,920	642	1,285	1,285	642	642
831	1	Greenhouse	Small ciliates	990,000	568,000	1,765,000	424,000	565,000	282,000	282,000	141,000	282,000	141,000
			Large ciliates	212,000	353,000	212,000	212,000	282,000	70,700	141,000	70,700	70,700	70,700
			Flagellates	1,000,000	381,000	41,800	13,900	14,000			70,700		141,000
832	5	…do…	Small ciliates	84,500	84,500	41,800	310,000	197,000	13,900	13,900	70,600	13,900	112,300
			Large ciliates	10,590	10,590	74,000	7,060	7,060	7,060	7,060	10,590	3,530	3,530
			Flagellates					13,900					
833	20	…do…	Small ciliates	7,060	7,060	3,530	7,060	7,060	10,590	3,530	3,530	7,060	7,060
			Large ciliates	1,390	1,390	4,170	1,390						
			Flagellates										
834	50	…do…	Small ciliates	4,170	1,390		4,170		4,170	38,300	235,000	4,170	1,390
			Large ciliates	642					1,390	2,033	3,530		
			Flagellates		1,390			2,780					
835	100	…do…	Small ciliates		1,390				642				
			Large ciliates										
			Flagellates	2,780	2,780	1,390	4,920	17,000	29,700	41,700	25,480	33,800	28,250

development, as only a few appeared with some of the inoculations on the seventh to the eleventh day after inoculation. In hay infusion, however, they began to appear on the third day; the maximum numbers were reached on the fifth to the sixth day after inoculation; then the numbers decreased.

DEVELOPMENT OF FLAGELLATES

That there were more flagellate cysts in the soils examined or that the media employed were more favorable for the development of these organisms than for ciliates is clearly seen by the fact that in all the culture solutions these organisms were more numerous. There was a great variation in the development of these organisms in the different solutions when inoculated with different soils. Upon noting the development of these organisms from field soil it is seen that in the dried-blood extract they appeared the first and second days after inoculation, the maximum number being reached on the third and fourth days. In hay infusion they appeared the second and third days; the maximum numbers were present from the sixth to the eighth day.

In inoculations of both media with greenhouse soil the flagellates appeared on the first day, the greatest numbers appearing from the second to the fourth day in the blood extract and from the third to the eleventh days in the cases of the inoculated solution of hay infusion. In comparing the period of maximum development of flagellates from the various compost soil inoculations, it is seen that with large amounts of soil inoculations the greatest development was attained several days before the maximum had been reached with the smaller inoculations. In total numbers of organisms developing there was no greater majority from either soil examined. In some solutions of dried-blood extract and hay infusion the greatest numbers were developed from field soils, while the greatest development in others was from greenhouse soil.

DEVELOPMENT OF ALL FORMS OF PROTOZOA

For the development of all forms of protozoa hay infusion was more favorable than dried-blood extract. In the former solutions the bacteria seemed to thrive and multiply much more readily than in the latter. Thus, if protozoa feed upon these organisms, favorable conditions for the development of bacteria might have stimulated protozoan life. In comparing the numbers of all forms of protozoa present it is seen that the maximum development is always present at an earlier period in the solutions with the largest inoculations. This is in all probability due to the presence of many more cysts in solutions to which larger amounts of soil were added.

In considering the development of protozoa on the gram-inoculation basis more than one hundred times as many organisms excysted from the smaller than the larger inoculations of soil. This fact may be due

to the increased toxicity produced from the decomposition products of bacteria and other organisms. It is very clearly noted that in all inoculated solutions as soon as the maximum development is reached there is a gradual decrease in numbers of all types of organisms. In so far as observations have been made, this fact is attributed to several causes: The soil contains various kinds and numbers of living organisms as well as spores and many cysts. If the temperature and other conditions, such as sufficient desirable food, the absence of harmful toxins, and the presence of favorable reactions (acidity or alkalinity) are present when a culture solution is inoculated with the soil, certain types of protozoa as well as other organisms excyst and multiply until conditions become unfavorable. Owing to the lack of desirable food for certain species of organisms, the presence of certain decomposition products of either bacteria, yeasts, molds, or protozoa, or the direct destruction by other forms, the organisms either encyst or die. The organisms probably remain inactive until conditions again become favorable, when they excyst and multiply as before.

TYPES OF PROTOZOA DEVELOPED IN DIFFERENT CULTURE SOLUTIONS

In hay-infusion solutions inoculated with the greenhouse soil the organisms observed corresponded to *Vorticella* spp., *Colpoda cucullus*, *Colpidium colpoda*, *Prorodon ovum* Ehrb., and *Glaucoma* sp. The first two mentioned appeared the second day after inoculation; the others were first noted on the sixth day. In some solutions a few of these forms were still present on the thirtieth day. No vorticellæ developed from any inoculations with field soil, indicating that this form inhabits rich moist soil. This fact has already been noted by several investigators. In hay infusion several different unidentified types developed. This has never before been observed in any of the other culture solutions. A large type of flagellates developed on the third day after inoculation. In some solutions of hay infusion these forms appeared soon after the smaller ones had disappeared. Colpoda was the most common form of ciliate in inoculations with field soil. From inoculations of field soil types of ciliates developed different from those in solutions to which greenhouse soil was added. It was also noted that fewer types of organisms were developed from the former than from the latter soil.

In hay infusion the large ciliates were markedly different from those which had already been observed with dried-blood extract. In the former solution inoculated with greenhouse soil but one type was observed. It was very apparent that dried-blood extract was the most favorable for the development of many types of large ciliates.

On the ninth day after the inoculation in hay infusion to which field soil had been added millions of very minute organisms that appeared to be flagellates were noted. These could barely be recognized under the low power of the microscope. This form was not counted, but was

observed to be present throughout the experiment. Similar protozoa appeared on the fourteenth day in hay infusion to which greenhouse soil had been added.

In hay infusion it was very apparent that as the numbers of protozoa decreased the number of cysts increased. That all the protozoa do not encyst when conditions become unfavorable is shown by the fact that very many dead forms of Glaucoma and Prorodon were seen.

On the twentieth day species of Euglena appeared in dried blood inoculated with field soil and in hay infusion to which greenhouse soil had been added. The latter solution was favorable for the development of these organisms, as 600 per gram of soil appeared on the twentieth day, while on the thirtieth day 28,000 organisms of *Euglena* spp. per gram were counted.

That the soils examined contained a small number of amœbæ cysts or that the conditions in the culture solutions were unfavorable for their development was judged by the fact that very few were observed. From the soil inoculations in dried blood no forms of amœbæ were recognized, while in the case of the hay infusion to which greenhouse soil had been added a few were observed on the twenty-first day.

SUMMARY OF PART III

Under the conditions of this experiment and of Part II it is apparent that in developing protozoa from the soil in artificial culture solutions different numbers and types of protozoa will be developed for every variation in the amounts of each soil used for inoculation and with every culture solution used.

IV.—EFFECT OF TEMPERATURE UPON THE DEVELOPMENT OF SOIL PROTOZOA

INTRODUCTION

In the earlier experiments recorded in Parts II and III of this article it was shown that the development of the numbers and types of soil protozoa in artificial culture solutions varied with the kind of culture solutions as well as with the quantity, physical condition, and kind of soil used for inoculation.

The problem under discussion deals with the development of the numbers and types of soil protozoa which appear at various temperatures in artificial culture solutions inoculated with soil of different origin.

That different conditions of temperature affect the development of protozoa in the soil was recorded by Cunningham (3, p. 14), who, after inoculating a quantity of soil for a period of nine days at 5 to 7° C., then increasing the incubation temperature to 22° C., noted an increase in the numbers of protozoa developed after seven days. "Exposure to a temperature of 30° C. for seven days has caused a fall in the total numbers but a distinct rise in the number of cysts."

A sample of the 20 per cent compost greenhouse soil which had been used in previous experiments was collected. Likewise another sample of the heavy unfertilized clay soil used in another study was brought to the laboratory. A third sample, a light loamy soil which contained 12.19 per cent of moisture and which had received an application of 20 tons of barnyard manure per acre for the last 20 years, was collected 3 inches below the surface. The upper 1¾ inches were frozen, but the temperature at 3 inches was 1.5° C. at the time of sampling.

Four 200 c. c. Jena Erlenmeyer flasks containing 100 c. c. portions each of 3 per cent dried-blood extract with 0.05 per cent of dibasic potassium phosphate and the same number of flasks containing 100 c. c. portions of a 10 per cent hay infusion were inoculated with 5-gm. samples of the newly collected moist soils. (It was found in Parts II and III that on the gram basis a greater development of protozoa can be produced with smaller amounts of soil.) The inoculated solutions were examined for living protozoa, and then one flask of each solution of hay infusion and blood extract inoculated with each soil was incubated at temperatures of 5 to 7° C., another set at 15 to 16° C., a third at 22 to 23° C., and a fourth at 29 to 30° C., for a period of 30 days. At the same hour each day these solutions were examined and the living protozoa counted by the improved loop method under the low power of the microscope.

In order that the inoculated solutions should not vary in temperature during examination, they were kept in constant-temperature baths. In like manner, to guard against excessive evaporation from the solutions inoculated at 29 to 30° C., each flask was placed in a container of water and covered with a large beaker. To prevent variation through the possible effect of excessive light, the flasks were screened in all cases during the incubation period.

As in the previous experiments, the classification of protozoa that was followed in this problem was as follows: The small ciliates included all organisms from the smallest to and including *Colpidium colpoda*. The vorticella type were also included. The large ciliates included all forms larger than *Colpidium colpoda*. The flagellates included all the forms of flagellates that were observed.

DEVELOPMENT OF SOIL PROTOZOA IN CULTURE SOLUTIONS AT VARIOUS TEMPERATURES

DEVELOPMENT OF SMALL CILIATES

That the varying condition of temperature is a very important factor in the development of soil protozoa in culture solutions is noted by the marked variation in the numbers of organisms present. For the development of small ciliates a temperature of 6 to 7° C. proved very unfavorable, as in none of the inoculated solutions did the organisms exceed 265,000 per gram. At this temperature blood extract seemed to be more

TABLE X.—*Number of small ciliates, large ciliates, and flagellates developed daily at various temperatures in different culture solutions inoculated with the same amount of different soils for a period of 30 days. These numbers have all been calculated on the basis of 1 gm. of dry soil*

Solution No.	Kind of soil	Medium	Temperature of incubation	Kind of organisms	1	2	3	4	5	6	7	8	9	10
1111	Greenhouse	Blood extract	6 to 7° C	Small ciliates										
				Large ciliates	15,500	15,590	15,500	77,500	930,000	373,000	652,000	3,500,000	4,400,000	15,200,000
				Flagellates										
1112	...do...	Hay infusion	...do...	Small ciliates										
				Large ciliates										
				Flagellates										
1113	Field	Blood extract	...do...	Small ciliates										
				Large ciliates					53,100	451,000	571,000	1,205,000	1,735,000	4,380,000
				Flagellates										
1114	...do...	Hay infusion	...do...	Small ciliates										
				Large ciliates							279,000	584,000	318,000	438,000
				Flagellates										
1115	Field+manure	Blood extract	...do...	Small ciliates		14,020		70,200	942,000	8,350,000	19,700,000	4,920,000	4,930,000	92,400,000
				Large ciliates										
				Flagellates										
1116	...do...	Hay infusion	...do...	Small ciliates					42,200	1,670,000	4,000,000	2,998,000	3,130,000	11,450,000
				Large ciliates										
				Flagellates										
1121	Greenhouse	Blood extract	15 to 16° C	Small ciliates	13,680									
				Large ciliates					27,450	96,000	178,000	13,700		
				Flagellates		13,680	136,800			13,700	13,700	27,400	178,000	123,200
1122	...do...	Hay infusion	...do...	Small ciliates		15,500			93,200	497,000				
				Large ciliates				7,150,000	3,770,000	2,425,000	412,000	404,000	497,000	435,000
				Flagellates			2,220,000	2,250,000	11,330,000	12,350,000	14,200,000	32,600,000	102,000,000	73,000
1123	Field	Blood extract	...do...	Small ciliates										
				Large ciliates										
				Flagellates			208,300	900,000	677,000	221,000	1,497,000	3,050,000	8,480,000	8,900,000
1124	...do...	Hay infusion	...do...	Small ciliates		14,820			12,340	12,340	61,700	37,020	37,100	37,100
				Large ciliates						24,680	118,700	178,000		
				Flagellates				1,097,000	3,560,000	2,580,000			32,600,000	6,650,000
1125	Field+manure	Blood extract	...do...	Small ciliates							24,680	86,380	173,000	161,000
				Large ciliates						27,300	14,050	337,000	147,000	83,400
				Flagellates		61,800	1,112,000	7,900,000	8,590,000	7,770,000	1,777,000	642,000	281,000	420,000
1126	...do...	Hay infusion	...do...	Small ciliates										
				Large ciliates										
				Flagellates			407,000	5,820,000,000	101,000,000	45,700,000	47,400,000	213,590,000	94,400,000	35,200,000
1131	Greenhouse	Blood extract	22 to 23° C	Small ciliates	25,300	50,600	88,500	202,000	63,200	25,300		12,650	12,620	12,620
				Large ciliates				79,800		12,600		12,650		
				Flagellates			872,000					25,300		

No.	Source	Temp.	Treatment	Organism											
1132	...do...		Hay infusion	Small ciliates				53,200	31,000,000	26,900,000	82,000,000	9,900,000	24,200,000	6,440,000	7,220,000
				Large ciliates				239,000	7,400,000	15,970,000	25,500,000	133,000	133,000	13,260	13,260
				Flagellates									266,000	360,000	1,448,000
1133	Field		Blood extract	Small ciliates					9,150,000	5,600,000	12,686,000	1,645,000	2,170,000	2,168,000	
				Large ciliates				12,200	6,150,000						440,000
				Flagellates						59,790	126,000	228,300	915,000	3,900,000	1,280,000
1134	...do...		Hay infusion	Small ciliates				988,000		27,900,000	9,150,000	790,000	736,000	1,370,000	279,500
				Large ciliates				11,550	92,800	81,000	46,400	11,500		11,550	
				Flagellates											
1135	Field+manure		Blood extract	Small ciliates	11,550				5,460,000	602,000	1,540,000	567,000	2,393,000	206,000	23,200
				Large ciliates	1,560,000				600,000	2,020,000	301,000	216,000	433,000	60,000	180,000
				Flagellates	6,060,000										
1136	...do...		Hay infusion	Small ciliates		108,000			16,900,000	236,000,000	100,500,000	6,240,000	6,400,000	432,000	1,584,000
				Large ciliates						12,600					
				Flagellates											
1141	Greenhouse	29 to 30° C.	Blood extract	Small ciliates	12,600	25,200	119,800	30,800,000	108,600,000	22,530,000	13,200,000	13,059,000	4,680,000	586,000	452,500
				Large ciliates							59,400	12,600	37,800	12,620	25,240
				Flagellates											
1142	...do...		Hay infusion	Small ciliates	53,200	26,600	3,810,000		5,850,000		1,223,000	930,000	5,270,000	173,000	39,900
				Large ciliates					12,180						12,200
				Flagellates											
1143	Field		Blood extract	Small ciliates		61,000	109,200						97,600	170,500	330,000
				Large ciliates											
				Flagellates											
1144	...do...		Hay infusion	Small ciliates					915,000	1,685,000	750,000	701,000	475,000	3,000,000	1,913,000
				Large ciliates					185,000	543,000	139,000	219,300	185,000	81,000	104,000
				Flagellates											
1145	Field+manure		Blood extract	Small ciliates	11,550				46,200	23,100	23,100	11,550	34,650	335,000	243,000
				Large ciliates	24,000				9,120,000	12,100,000	26,250,000	5,480,000	288,000	106,000	72,000
				Flagellates											
1146	...do...		Hay infusion	Small ciliates		12,000			33,700,000	13,600,000	5,620,000	1,655,000	168,000	377,000	36,000
				Large ciliates											
				Flagellates											

TABLE X.—*Number of small ciliates, large ciliates, and flagellates developed daily at various temperatures in different culture solutions inoculated with the same amount of different soils for a period of 30 days. These numbers have all been calculated on the basis of 1 gm. of dry soil*—Continued

Solution No.	Kind of soil	Medium	Temperature of incubation	Kind of organisms	11	12	13	14	15	16	17	18	19	20
1111	Greenhouse	Blood extract	6 to 7° C	Small ciliates		31,000		93,000	15,500	77,600	155,200	77,600	46,600	15,500
				Large ciliates				15,500	15,500	15,500	15,500	15,500		
				Flagellates	32,186,000	19,800,000	13,320,000	20,600,000	11,240,000	2,140,000	3,650,000	947,000	248,000	792,000
1112	...do...	Hay infusion	...do...	Small ciliates										
				Large ciliates										
				Flagellates										
1113	Field	Blood extract	...do...	Small ciliates	7,950,000	10,740,000	17,550,000	15,559,000	47,100,000	52,900,000	48,750,000	17,400,000	20,100,000	35,400,000
				Large ciliates										
				Flagellates										
1114	...do...	Hay infusion	...do...	Small ciliates	982,000	372,000	119,500	398,500	93,000,000	39,500	584,000	6,550,000	11,460,000	15,220,000
				Large ciliates				13,650	13,650		13,650	13,650	13,650	28,000
				Flagellates										
1115	Field+manure	Blood extract	...do...	Small ciliates	12,540,000	520,000	266,300	343,000	687,500	590,000	1,193,000	1,430,000	872,000	2,635,000
				Large ciliates										
				Flagellates										
1116	...do...	Hay infusion	...do...	Small ciliates	3,650,000	844,000	14,959,000	42,300,000	24,200,000	11,820,000	59,600,000	26,000,000	66,000,000	61,300,000
				Large ciliates										
				Flagellates										
1121	Greenhouse	Blood extract	15 to 16° C	Small ciliates	6,859,000	27,400	356,000	1,186,000	850,000	493,000	41,100	13,680	13,700	27,300
				Large ciliates	404,000	1,586,000	2,200,000	902,000	232,500	590,000	295,000	186,000	105,500	201,500
				Flagellates										
1122	...do...	Hay infusion	...do...	Small ciliates	65,300,000	70,200,000	95,500,000	32,200,000	51,700,000	57,900,000	27,800,000	15,960,000	14,250	761,000
				Large ciliates	26,600	26,600	104,000	79,800	39,100	39,100	13,000	52,000	13,000	26,000
				Flagellates										
1123	Field	Blood extract	...do...	Small ciliates	18,550,000	19,750,000	27,400,000	14,370,000	6,300,000	8,320,000	7,760,000	352,000	456,000	195,100
				Large ciliates	118,600	148,000	118,600	714,000	1,066,000	13,900,000	14,100,000	43,300,000	45,400,000	8,700,000
				Flagellates										
1124	...do...	Hay infusion	...do...	Small ciliates	187,500,000	21,700,000	12,800,000	104,000,000	40,600,000	10,120,000	27,600,000	38,800,000	30,800,000	8,350,000
				Large ciliates	24,720	24,720	24,720	12,360	12,360				24,700	12,320
				Flagellates	123,700									12,360
1125	Field+manure	Blood extract	...do...	Small ciliates	468,000	370,000	49,400	49,400	12,330	24,720	24,720	24,720	61,800	61,800
				Large ciliates	19,100,000	9,620,000	6,210,000	3,090,000	1,990,000	422,000	309,800	575,000	1,095,000	337,000
				Flagellates										
1126	...do...	Hay infusion	...do...	Small ciliates	293,000,000	210,000,000	5,780,000	20,380,000	1,333,000	4,859,000	14,000	182,500	506,000	126,300
				Large ciliates										
				Flagellates										
1131	Greenhouse	Blood extract	22 to 23° C	Small ciliates										
				Large ciliates										
				Flagellates		12,620	12,620	12,620					12,620	

No.	Source	Medium	Temp.	Organism	6,150,000	4,950,000	4,940,000	4,340,000	2,300,000	3,600,000	3,940,000	2,980,000	1,143,000	412,500
1132	...do...	Hay infusion		Small ciliates	53,200		13,260	13,260	13,260	13,260	13,260	13,260	13,260	
				Large ciliates	625,000	39,950	26,520	26,520						26,600
				Flagellates				12,180						
1133	Field	Blood extract		Small ciliates	513,000	256,000	122,000	146,200	146,200	195,000	134,000	121,000	24,000	48,800
				Large ciliates	1,886,000	1,624,000	1,700,000	800,000	559,000	241,000	420,000	89,000	101,500	139,500
				Flagellates										
1134	...do...	Hay infusion		Small ciliates	76,200	12,700	12,700	38,100			25,400			
				Large ciliates		11,550	11,550				11,550			
				Flagellates										
1135	Field+manure	Blood extract		Small ciliates	81,000	625,000	196,500	370,000	10,350,000	1,320,000	5,370,000	660,000	680,000	58,000
				Large ciliates	192,200	24,000	36,000	12,000	12,000	12,000	24,000	24,000	24,000	24,100
				Flagellates										
1136	...do...	Hay infusion		Small ciliates	6,340,000	1,271,000	830,000	815,000	747,000	2,283,000	288,000	168,000	264,400	60,000
				Large ciliates										
				Flagellates										
1141	Greenhouse	Blood extract	29 to 30° C.	Small ciliates	12,620	12,620	25,240	12,620	12,620					
				Large ciliates	50,480	37,860			37,860	25,240	106,500	53,250	25,240	139,000
				Flagellates	4,030,000	53,250	80,000	40,000	26,600	53,250			13,300	79,800
1142	...do...	Hay infusion		Small ciliates		39,900			13,300	13,300	13,300	13,300	26,600	26,600
				Large ciliates	12,200	12,200	24,400	12,200	36,600	24,400	12,200	12,200	12,200	12,200
				Flagellates										
1143	Field	Blood extract		Small ciliates	207,000	109,000		12,200	24,400	36,600	12,200			12,200
				Large ciliates				88,900						
				Flagellates										
1144	...do...	Hay infusion		Small ciliates	1,687,000	533,000	59,800		240,600	12,700	25,400		12,700	12,700
				Large ciliates	69,500	38,000	58,000	46,300	23,159	23,159	23,159	23,150		
				Flagellates										
1145	Field+manure	Blood extract		Small ciliates	57,800	104,000	23,150	11,570	69,500	46,300	46,300	34,750	104,000	11,500
				Large ciliates	60,000	12,000	24,000	12,000	12,000					12,000
				Flagellates										
1146	...do...	Hay infusion		Small ciliates	24,000			12,000	12,000					
				Large ciliates										
				Flagellates										

TABLE X.—Number of small ciliates, large ciliates, and flagellates developed daily at various temperatures in different culture solutions inoculated with the same amount of different soils for a period of 30 days. These numbers have all been calculated on the basis of 1 gm. of dry soil—Continued

Solution No.	Kind of soil	Temperature of incubation	Medium	Kind of organisms	Days after inoculation									
					21	22	23	24	25	26	27	28	29	30
1111	Greenhouse	6 to 7° C.	Blood extract	Small ciliates	31,000	15,500	15,500		15,500					
				Large ciliates	15,500	15,500			31,000					
				Flagellates	1,440,000	155,000	948,000	575,000	1,147,000	1,426,000	46,600			263,200
1112	....do		Hay infusion	Small ciliates							77,800	93,200	622,000	
				Large ciliates							119,200	39,800		26,500
				Flagellates										
1113	Field		Blood extract	Small ciliates	11,000,000	2,680,000	1,800,000	504,000	278,200	412,000	199,000	79,600	225,000	66,400
				Large ciliates										
				Flagellates										
1114	....do		Hay infusion	Small ciliates										
				Large ciliates					56,000	28,000	70,300	28,000	70,300	14,000
				Flagellates	21,670,000	53,400,000	66,500,000	21,940,000	108,800,000	25,080,000	82,400,000	134,600,000	74,800,000	106,500,000
1115	Field+manure		Blood extract	Small ciliates										
				Large ciliates		42,200	14,000							
				Flagellates	1,404,000	450,000	1,837,000	772,500	1,010,000	870,000	42,200	2,160,000	618,000	518,000
1116	....do		Hay infusion	Small ciliates										
				Large ciliates										
				Flagellates	97,000,000	59,800,000	55,600,000	33,600,000	35,300,000	61,800,000	53,200,000	53,650,000	73,000,000	95,500,000
1121	Greenhouse	15 to 16° C.	Blood extract	Small ciliates	13,650	13,650	27,300	13,650	27,300	13,650	27,300	13,650	27,300	27,300
				Large ciliates		13,650	54,000	13,650	13,650	13,650	54,600	40,950	54,600	54,600
				Flagellates	1,118,000	885,000	232,500	1,740,000	201,500	388,000	450,000	278,500	404,000	263,400
1122	....do		Hay infusion	Small ciliates	139,500	482,000	171,000	46,700	39,000	15,500	62,200	46,500	46,500	
				Large ciliates	26,000	26,000	39,000	26,000		13,000	26,000	39,000	26,000	
				Flagellates										
1123	Field		Blood extract	Small ciliates			13,000	13,000	91,000	52,000	39,000	78,000	78,000	65,000
				Large ciliates										
				Flagellates	862,000	4,880,000	9,110,000	4,570,000	1,216,000	1,350,000	460,000	1,305,000	1,274,000	1,335,000
1124	....do		Hay infusion	Small ciliates	24,640	166,500	505,000	49,400	59,400	14,800	14,800	29,600	356,500	312,000
				Large ciliates										
				Flagellates	550,000	1,230,000		89,200	8,880,000	8,280,000	5,480,000	5,160,000	5,550,000	135,500
1125	Field+manure		Blood extract	Small ciliates	210,000	24,720	729,000	99,000	280,000	98,500	24,720	37,000	172,500	24,720
				Large ciliates	210,400	547,000	420,800	280,000			238,800	56,150	295,000	399,000
				Flagellates										
1126	....do		Hay infusion	Small ciliates										
				Large ciliates										
				Flagellates	252,500	604,000	5,880,000	2,272,000	13,050,000	9,890,000	6,770,000	6,950,000	1,395,000	478,000
1131	Greenhouse	22 to 23° C.	Blood extract	Small ciliates	12,620	25,240	12,620	12,620	12,620	37,860	25,240	12,620	25,240	12,620
				Large ciliates										
				Flagellates										12,620

No.	Sample	Substrate	Temp.	Organism													
1132	....do	Hay infusion	....do	Small ciliates	718,000	680,000	758,000	379,000	172,500	49,000	26,600	26,600					
				Large ciliates	13,300	13,300			13,300	13,300		13,300	13,300	13,300		13,300	
				Flagellates	13,300	26,600			13,300	13,300		13,300			39,900	26,600	
1133	Field	Blood extract	....do	Small ciliates													
				Flagellates	61,000	36,600	12,200	127,000	12,200	24,400	24,400	24,400	24,400	24,400	24,400	
				Large ciliates	76,200	101,300	101,300	127,000	165,000	88,800	88,800	127,000	152,000	139,500	
1134	....do	Hay infusion	....do	Small ciliates			12,700		12,700	25,400	25,400	23,120	12,700	12,700	59,800
				Large ciliates			11,560		23,120	34,730	11,560	11,560		23,120	
				Flagellates											
1135	Field+manure	Blood extract	....do	Large ciliates	254,400	23,120	11,560	150,300	23,120	231,200	127,000	127,000	127,000	115,600	69,400
				Small ciliates	12,950		12,950	12,950	23,120			24,100			12,950
				Flagellates											
1136	....do	Hay infusion	....do	Small ciliates	36,070	72,140	132,000	96,200	252,300	613,000	1,958,000	337,000	2,030,000	204,000
				Large ciliates										
				Flagellates		12,600					12,600			12,600
1141	Greenhouse	Blood extract	29 to 30° C.	Small ciliates	12,600	75,600	12,600		37,800	25,200		25,200	25,200	
				Large ciliates	146,100	40,000	13,300	26,600	40,000	26,600	79,800		13,300
				Flagellates	13,300	13,300	13,300						13,300
1142	....do	Hay infusion	....do	Small ciliates	26,600	13,300	13,300	13,300	39,900	53,200	13,300	13,300	26,600	53,200
				Large ciliates	12,200	12,200	12,200	24,400	12,200	12,200	39,900	280,000
				Flagellates								
1143	Field	Blood extract	....do	Small ciliates	12,200	12,200	24,400		12,200	12,200		24,400	24,400	24,400
				Large ciliates								
				Flagellates								
1144	....do	Hay infusion	....do	Small ciliates		12,700			34,650	12,700	12,700		12,700
				Large ciliates								
				Flagellates								
1145	Field+manure	Blood extract	....do	Large ciliates	23,000	34,500	11,500	57,500	57,500	23,000	11,500	11,500	23,000	23,000
				Small ciliates	12,000	96,000	12,000	72,000	36,000	24,000	24,000	24,000	24,000
				Flagellates								
1146	....do	Hay infusion	....do	Large ciliates					12,000	12,000		
				Flagellates								

favorable than hay infusion. That the conditions in the solutions examined were unfavorable for the existence of these organisms is seen by the fact that they did not appear until the twelfth to the fourteenth day, while in others they did not develop throughout the entire period of 30 days.

With the exception of solutions of hay infusion inoculated with greenhouse and fertilized field soil, in all inoculations of both media greater numbers of small ciliates developed at 15° to 16° C. than at any other temperature, indicating that with these culture solutions this temperature is the most favorable for the development of small ciliates. With every soil examined there was a much greater development of these organisms in hay infusion than in blood extract. These facts indicate that only certain types of small ciliates exist and develop at a definite temperature and that the extent of their development is influenced by the amount of desirable food and other favorable conditions in the culture solutions. Because of the fact that the development of small ciliates in dried-blood extract at 15° to 16°, 22° to 23°, and 29° to 30° C. was not as great as in the solutions of hay infusion, the maximum numbers were in most cases developed sooner in the former than in the latter solutions. At 15° to 16° C. in inoculated solutions of both media the period of maximum development varied considerably. In the inoculation of dried-blood extract with greenhouse soil the greatest numbers were reached on the seventh day, while with the other soils a longer time was required. The maximum development with the field-soil inoculations was on the thirteenth day. With the fertilized field soil the greatest number was not reached until the twenty-fifth day after inoculation. In solutions of hay infusion with greenhouse soil the maximum number of organisms was reached on the thirteenth day, with the fertilized field soil on the eleventh day, and with the field soil on the nineteenth day after inoculation.

Incubating at higher temperatures as a rule encouraged a more rapid development of these organisms. However, this fact was not universal, but it was noted that at the temperatures of 22° to 23° and 29° to 30° C. the small ciliates began to appear at an earlier period than at lower temperatures of inoculation. This fact is especially marked in the greenhouse and fertilized-soil inoculations of both media. At 29° to 30° C. in the greenhouse-soil inoculations the organisms appeared on the second day, at 22° to 23° C. on the third day, at 15° to 16° C. on the fifteenth, and at 6° to 7° C. in one inoculation on the twelfth day, and with the other they did not appear until the twenty-seventh day. A very similar circumstance was noted with the fertilized field soil, but in this case at the highest temperature the organisms did not appear until the third day, in solutions inoculated at 15° to 16° C. on the fifth to sixth day, while in solutions inoculated at 6° to 7° C. they appeared on the four-

teenth day, and in the other solution they did not appear at all. For the development of the greatest numbers of small ciliates at the different temperatures, in the media inoculated with the various soils, the greatest numbers developed in hay infusion at 29° to 30° C. with inoculations of greenhouse and manured field soil, while with field soil the maximum development was at 15° to 16° C.

DEVELOPMENT OF LARGE CILIATES

On examining Tables X, XI, and XII it at once becomes apparent that either the soils examined contained very few large ciliate cysts or the conditions in the media were unfavorable for their existence, as very few of these organisms developed. Blood extract seemed a little more favorable than hay infusion. In all the inoculations with the greenhouse soil a lew large ciliates developed. In solutions of dried-blood extract with the greenhouse soil at 6° to 7° they first appeared on the fourteenth day, at 15° to 16° on the sixth, at 22° to 23° on the fourth, and at 29° to 30° C. they did not appear until the ninth day after inoculation. At 22° to 23° and 29° to 30° C. a few large ciliates developed in the hay-infusion inoculations of greenhouse soil. With the exception of a few organisms that appeared in the blood-extract inoculations of the manured field soil at 15° to 16° and in the hay-infusion inoculations of field soil at 29° to 30° C. and in the one mentioned above, no large ciliates appeared in any of the solutions.

DEVELOPMENT OF FLAGELLATES

Again the temperature plays a very important rôle in the development of flagellates. Upon considering the largest numbers of organisms developed at different temperatures in dried-blood extract, it is seen that in all cases the greatest development appeared at 6° to 7° and the smallest at 29° to 30° C. There were 150 to 250 times as many developed at the former temperature as at the latter. In comparing all inoculations of hay infusion it is noted that the greatest development for all the soils examined was at 15° to 16°; the smallest in some cases occurred at 6° to 7°; in others at 29° to 30°. With one exception, at 6° to 7°, greater numbers were developed in hay infusion than in dried-blood extract. Comparing the greatest numbers of flagellates developed at the different temperatures and in the various media, it is seen that the greatest development for all soils was in hay infusion at 15° to 16° C. In all cases in the inoculations of hay infusion and in most cases with dried-blood extract at 6° to 7° these organisms did not appear until the fifth day and the maximum was not reached until the twenty-eighth or twenty-ninth day after inoculation.

TABLE XI.—*Period of the maximum development and number of small ciliates, large ciliates, and flagellates at different temperatures in dried-blood extract and hay infusion inoculated with the same amount of the various soils*

SMALL CILIATES

Soil	Incubated at 6° to 7° C. — Dried-blood extract	Days after inocu-lation	Incubated at 15° to 16° C. — Dried-blood extract	Days after inocu-lation	Incubated at 22° to 23° C. — Dried-blood extract	Days after inocu-lation	Incubated at 29° to 30° C. — Dried-blood extract	Days after inocu-lation	Incubated at 6° to 7° C. — Hay infusion	Days after inocu-lation	Incubated at 15° to 16° C. — Hay infusion	Days after inocu-lation	Incubated at 22° to 23° C. — Hay infusion	Days after inocu-lation	Incubated at 29° to 30° C. — Hay infusion	Days after inocu-lation
Greenhouse	155,200	17	178,000	7	88,500	3	75,800	3	263,200	30	2,200,000	13	82,000,000	6	108,600,000	4
Field	119,200	27	104,000	13	12,180	14	36,600	15	....	....	45,400,000	19	3,900,000	9	88,900	14
Field + manure	70,300	27	8,880,000	25	92,800	4	543,000	5	....	....	19,100,000	11	2,020,000	5	26,250,000	6

LARGE CILIATES

Soil	Incubated at 6° to 7° C. — Dried-blood extract	Days after inocu-lation	Incubated at 15° to 16° C. — Dried-blood extract	Days after inocu-lation	Incubated at 22° to 23° C. — Dried-blood extract	Days after inocu-lation	Incubated at 29° to 30° C. — Dried-blood extract	Days after inocu-lation	Incubated at 6° to 7° C. — Hay infusion	Days after inocu-lation	Incubated at 15° to 16° C. — Hay infusion	Days after inocu-lation	Incubated at 22° to 23° C. — Hay infusion	Days after inocu-lation	Incubated at 29° to 30° C. — Hay infusion	Days after inocu-lation
Greenhouse	31,000	25	13,700	6	70,800	4	12,620	9	....	....	....	....	53,200	11	26,600	29
Field	....	....	....	....	....	....	....	....	....	....	....	....	....	....	12,700	27
Field + manure	....	....	420,000	10	....	....	....	....	....	....	....	....	....	....	....	....

FLAGELLATES

Soil	Incubated at 6° to 7° C. — Dried-blood extract	Days after inocu-lation	Incubated at 15° to 16° C. — Dried-blood extract	Days after inocu-lation	Incubated at 22° to 23° C. — Dried-blood extract	Days after inocu-lation	Incubated at 29° to 30° C. — Dried-blood extract	Days after inocu-lation	Incubated at 6° to 7° C. — Hay infusion	Days after inocu-lation	Incubated at 15° to 16° C. — Hay infusion	Days after inocu-lation	Incubated at 22° to 23° C. — Hay infusion	Days after inocu-lation	Incubated at 29° to 30° C. — Hay infusion	Days after inocu-lation
Greenhouse	32,180,000	11	7,150,000	4	872,000	3	139,000	20	622,000	29	102,000,000	9	25,500,000	6	5,850,000	4
Field	52,900,000	16	27,400,000	13	12,680,000	6	330,000	10	134,600,000	28	187,500,000	11	27,900,000	5	3,000,000	9
Field + manure	92,400,000	10	8,540,000	5	10,350,000	15	335,000	9	97,000,000	21	293,000,000	11	236,000,000	5	33,700,000	4

DEVELOPMENT OF SOIL PROTOZOA AT DIFFERENT TEMPERATURES

From the data presented it is seen that the maximum number of small ciliates in the dried-blood extract were found at 15° to 16° C., while the greatest number of flagellates appeared at 6° to 7° C. In hay infusion the flagellates developed in greater numbers at 15° to 16°. In some solutions of hay infusion the small ciliates developed at 15° to 16° and in others at 29° to 30° C.

That the ciliates were directly detrimental to the development of flagellates can not be definitely stated at this time, but it is noted that in solutions of culture media incubated at 6° to 7° C., where very few ciliates developed when the flagellates had excysted, many more were present until the end of the experiment than had been noticed in any other inoculations. In view of this fact the flagellate development might be influenced by the presence of ciliates. At 6° to 7° C. the maximum number of flagellates appeared from 7 to 17 days before the maximum ciliate development was reached. At this temperature in no case was the maximum ciliate development noted until the tenth or eleventh day, while the greatest ciliate development appeared from the seventeenth to the twenty-seventh day after inoculation. In all inoculations of hay infusion at 6° to 7° the maximum number of flagellates developed at an earlier period than did the ciliates. The maximum development of the former occurred from the twenty-first to the twenty-ninth day, while that of the latter was 30 days after inoculation. In all inoculations at 15° to 16° the maximum development of small ciliates and flagellates was reached sooner than at the temperature of 6° to 7° C. This period varied from 2 to 20 days. In comparing the maximum numbers of small ciliates and flagellates developed at 22° to 23° with those developed at 15° to 16° it is seen that, with two exceptions, the greatest development was reached earlier at 22° to 23° C. Comparing the development at 22° to 23° with that at 29° to 30°, it is seen that there was no uniformity; in some cases the maximum was reached sooner at the former, while in others it occurred at the latter temperature. As in previous experiments, when the maximum development was reached, the numbers of protozoa gradually decreased, showing that the conditions for continuous multiplication became unfavorable.

Table XII shows the variation in the rate of multiplication of the protozoa in the different solutions from their first appearance until the first increase was noted. This increase was usually the first day after their presence was noted. There is a marked difference in the rate of multiplication of each type in each solution at every different temperature employed. No correlation is noted between the multiplication of the small ciliates in like infusions inoculated with the same soil incubated at different temperatures. The same statement holds true for the multiplication of the flagellates and large ciliates. In like

manner there is no correlation between the multiplication of the different protozoa (all types) in the different infusions inoculated with the same soil and incubated at the same temperature. For nearly all inoculations the greatest multiplication of the flagellates is noted at 22° to 23° C. In some solutions the small ciliates multiplied faster at 15° to 16°, in others at 22° to 23°, and in still others at 29° to 30° C.

TABLE XII.—*Rate of multiplication of the protozoa in culture solutions from the time of their first appearance until the first increase was noted. This period was usually 24 hours*

INCUBATED AT 6 TO 7° C.

Solution No.	Large ciliates.	Small ciliates.	Flagellates.
1111	2. 0	3. 0	5. 0
1112	....	6. 0	1. 2
1113	....	0	9. 0
1114	....	....	2. 0
1115	....	2. 0	13. 0
1116	....	....	40. 0

INCUBATED AT 15 TO 16° C.

1121	0	4. 0	10. 0
1122	....	5. 0	148. 0
1123	....	2. 0	4. 5
1124	....	1. 5	3. 0
1125	2. 0	2. 0	18. 0
1126	....	24. 0	14. 0

INCUBATED AT 22 TO 23° C.

1131	0	2. 3	2. 0
1132	4. 0	588. 0	3. 0
1133	....	0	82. 0
1134	....	2. 5	4. 5
1135	....	8. 0	136. 0
1136	....	3. 3	1,408. 0

INCUBATED AT 29 TO 30° C.

1141	0	6. 0	2. 0
1142	2. 0	259. 0	2. 0
1143	....	2. 0	1. 5
1144	0	0	2. 0
1145	....	3. 0	4. 0
1146	....	380. 0	401. 0

If in the future study of soil protozoa quantitative determinations are to be made, definite standards of quantity and of certain character (moist or dry) of soil must be taken in order that all the results will be comparable. Likewise, a definite standard of inoculation temperature and the kind of culture media must be fixed. The time at which the examinations are made must be uniform.

In so far as the data presented in Parts II and III show on the gram basis the greatest development of certain types of small ciliates, large ciliates, and flagellates is secured from 1-gm. inoculations in 100 c. c. of solution. However, 5 gm. of soil would be more representative than 1 gm; and, as shown by Cauda and Sangiorgi (2), on the gram basis

larger numbers of organisms would develop from this quantity than from larger amounts of soil.

In comparing the development of protozoa from moist and dry soils (Part II) very little difference in the development of small ciliates, large ciliates, and flagellates was found, but in order that the conditions of the soil protozoa would not be varied in this experiment moist soil was used.

Cunningham (3, p. 14) found 22° C. the optimum temperature for the development of most soil protozoa. In this problem a great variation was found. The greatest uniformity of results in the development of small ciliates was obtained at 15° to 16°. With flagellates 6° to 7° was the optimum in some solutions and 15° to 16° in others. As compared with the development at 22° to 23°, a much longer period of time was required for the maximum numbers of protozoa to develop at the temperatures lower than 22° to 23°. It may be added that 22° is more convenient to maintain in the laboratory.

In culture solutions the conditions are much different compared to those found in the soil, as was already suggested by Martin (12). Of the solutions examined the hay infusion proved the most satisfactory for the development of large numbers of organisms, the total being much greater than those appearing in the natural soil. It is probable that the results produced by adding the soil to tap water more nearly represents the conditions as they are found in the soil.

In order that the results may be comparable, the examinations of the inoculated culture must be made periodically every day. The length of time a culture should be incubated will vary with the temperature of incubation, kind and amount of soil, and kind of medium. Under certain conditions some types of protozoa appear on the first day after inoculation; these would multiply very rapidly and probably depress the development of other forms. Again, certain types might not appear until the eighteenth or twenty-second day after inoculation. With few exceptions, however, at temperatures of 15°, 22°, and 29° C., the maximum development of small ciliates and flagellates in the culture solutions inoculated with the soils examined was not reached until the thirteenth or fourteenth day. The writer is of the opinion that the development in artificial culture solutions in the first five to seven days would more nearly show the numbers of cysts of small ciliates and flagellates present in the soil.

The conditions under which the experiments have been carried out seemed to have been very unfavorable for the development of large ciliates and amœbæ. In the soils examined, however, cysts of large ciliates could be readily seen under the microscope. Cauda and Sangiorgi (2, p. 396) developed many amœbæ in Giltay's, Omelianski's, Hiltner's, peptone, and mannite solutions, showing that these organisms were present in the soils which they examined.

DEVELOPMENT OF THE DIFFERENT TYPES OF SOIL PROTOZOA

That the temperature influences the development of the different types of soil protozoa in culture solutions is shown by the fact that the numbers of the different species developed varied a great deal. The forms studied tallied best with the descriptions of *Colpoda cucullus*, *Colpidium colpoda*, *Vorticella* spp., *Prorodon ovum*, and *Glaucoma* sp. They appeared in some of each series of solutions incubated at 15°, 22°, and 29°. Of these organisms *Colpoda cucullus* was the only one that developed at 6°. At this temperature only a few appeared on the fourteenth day, and these were present until the twentieth day. Other ciliates that developed at 6° were *Paramecia* spp. At 15° *Colpoda cucullus* was the most numerous form of the ciliate type. These first appeared on the sixth day and were present throughout the experiment. Incubating at 15° was not very favorable for the development of the vorticellæ. They appeared on the fifth day and were still seen on the thirtieth day after incubation. Besides species of Colpoda, Vorticella, Prorodon, and Glaucoma, long slender ciliates, possibly *Condylostoma patens* Müll., were very numerous at 22° and 29°. At 22° species of Vorticella, Colpoda, Prorodon, and Glaucoma appeared on the third day. The vorticellæ were present until the twenty-sixth day after inoculation, while the other forms were still seen on the thirtieth day. The temperature of 29° was more favorable for the development of *Colpoda cucullus*, *Prorodon ovum*, and *Glaucoma* sp., but at this temperature many vorticellæ were also developed. This last-named form appeared on the second day and was noted until the fifteenth day after inoculation. The most prominent flagellates were species of Monas and Bodos.

As previously noted in Part III, all the small ciliates do not encyst when the conditions become unfavorable. This was again noted, for in the solutions incubated at 29° many dead individuals of *Prorodon ovum* and *Glaucoma* sp. were seen on the seventh day after inoculation.

The higher temperatures of incubation were the most favorable for the early excystment of small ciliates.

That the Vorticella cysts are present in field soils which have received applications of manure is shown by the fact that these organisms developed in culture solutions inoculated with the fertilized soil. The numbers, however, were very small as compared to those developed in solutions inoculated with greenhouse soil.

Upon several occasions during the examination of the solutions an individual of *Colpoda cucullus* was seen in the process of being excysted.

The conditions under which this experiment was carried out seemed to be unfavorable for the development of amœbæ, as none of these organisms developed in any of the media employed.

SUMMARY OF PART IV

Under the experimental conditions described above it has been found that:

(1) A temperature of 15° to 16° C. is the most favorable for the development of small ciliates.

(2) At 15° to 16° hay infusion is the most favorable culture solution for the development of small ciliates.

(3) The maximum numbers of small ciliates are developed at an earlier period in dried blood than in hay infusion.

(4) The maximum development of small ciliates at 6° to 7° varies from 17 to 30 days after inoculation.

(5) At 15° to 16° the maximum development of small ciliates occurs from seventh to twenty-fifth day after inoculation.

(6) The small ciliates develop sooner at the higher than at the lower temperatures.

(7) The lower temperatures retard the development of small ciliates.

(8) Dried-blood extract and hay infusion are unfavorable media for the development of large ciliates.

(9) Large ciliates will develop at all the temperatures noted if conditions are favorable.

(10) Many flagellates developed at all temperatures noted.

(11) The maximum development of flagellates occurs at 6° to 7° in dried-blood extract and at 15° to 16° in hay infusion.

(12) Hay infusion is the most favorable medium for the development of maximum numbers of flagellates.

(13) The higher temperatures encourage early development of flagellates, while lowest temperatures retard their development.

(14) At all temperatures the flagellates develop sooner than the ciliates.

(15) At 15° to 16° the flagellates appear four or five days before the ciliates.

(16) As soon as the maximum development is reached there is a gradual decrease in the numbers of all forms of protozoa.

(17) Species of Colpoda, Vorticella, Prorodon, and Glaucoma develop at 15° to 16°, at 22° to 23°, and at 29° to 30°.

(18) A few individuals of Colpoda and Paramecium develop at 6° to 7°.

(19) At 15° to 16° C. Colpoda is the most numerous ciliated form.

(20) The temperature of 29° to 30° is very favorable for the development of species of Colpoda, Prorodon, and Glaucoma.

(21) Some ciliates die when conditions become unfavorable.

(22) Vorticella cysts are present in field soils which have received applications of manure.

(23) Hay infusion and dried-blood extract are unfavorable media for the development of amœbæ.

GENERAL CONCLUSIONS ON THE DEVELOPMENT OF SOIL PROTOZOA IN ARTIFICIAL CULTURE SOLUTIONS

From all data which have been presented in this paper the writer feels that he is warranted in concluding that—

(1) The development of soil protozoa in artificial culture solutions inoculated with the soils which have been examined varies with the conditions of the problem.

(2) The development of soil protozoa in artificial culture solutions varies with the kind of media employed.

(3) For each quantity of soil which is used for inoculation there is a variation in the development of protozoa.

(4) Drying the soil affects the development of soil protozoa.

(5) There is a variation in protozoan development in every greenhouse soil.

(6) Field soil causes differences in the protozoan development.

(7) At each temperature of incubation there is a variation in the development of soil protozoa.

LITERATURE CITED

(1) BRÉAL, E.
    1896. Décomposition des matières végétales en présence de l'eau et de la terre. *In* Ann. Agron., t. 22, no. 8, p. 362–375.

(2) CAUDA, A., and SANGIORI, G.
    1914. Untersuchungen über die Mikrofauna der Böden aus Reisgegenden. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 42, No. 15/16, p. 393–398.

(3) CUNNINGHAM, Andrew.
    1914. Studies on soil protozoa. II. Some of the activities of protozoa. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 42, No. 1/4, p. 8–27.

(4) ——— and LÖHNIS, Felix.
    1914. Studies on soil protozoa. I. The growth of protozoa on various media and the effect of heat on active and encysted forms. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 39, No. 23/25, p. 596–610.

(5) EHRENBERG, C. G.
    1837. Die Fossilen Infusorien und die Lebendige Dammerde. 27 p., 2 col. pl., 1 tab. Berlin.

(6) FRANCÉ, R. H.
    1911. Studien über edaphische Organismen. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 32, No. 1/2, p. 1–7.

(7) GOODEY, T.
    1911. A contribution to our knowledge of the protozoa of the soil. *In* Proc. Roy. Soc. [London], s. B, v. 84, no. 570, p. 165–180, 1 fig., pl. 4.

(8) ———
    1914. Investigations on the protozoa of soil. (Abstract.) *In* Rpt. 83d Meeting Brit. Assoc. Adv. Sci., 1913, p. 775.

(9) GREEFF, Richard.
    1866. Ueber einige in der Erde lebende Amöben und andere Rhizopoden. *In* Arch. Mikros. Anat., Bd. 2, p. 299–331, pl. 17–18.

(10) KILLER, J.
    1913. Die Zählung der Protozoën im Boden. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 37, No. 17/21, p. 521–534.

(11) Löhnis, Felix.

 1911. Landwirtschaftlich-bakteriologisches Praktikum ... 156 p., 39 fig., 3 pl. Berlin.

(12) Martin, C. H.

 1913. The presence of protozoa in soils. *In* Nature, v. 91, no. 2266, p. 111.

(13) ——— and Lewin, K. R.

 1914. Some notes on soil protozoa. *In* Phil. Trans. Roy. Soc. London, s. B, v. 205, p. 77–94, pl. 5–6.

(14) Müller, P. T.

 1912. Über eine neue, rasch arbeitende Methode der bakteriologischen Wasseruntersuchung und ihre Anwendung auf die Prüfung von Brunnen und Filterwerken. *In* Arch. Hyg., Bd. 75, Heft 4/5, p. 189–223, 2 fig.

(15) Rahn, Otto.

 1913. Methode zur Schätzung der Anzahl von Protozoën im Boden. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 36, No. 15/18, p. 419–421.

(16) Russell, E. J., and Hutchinson, H. B.

 1909. The effect of partial sterilization of soil on the production of plant food. *In* Jour. Agr. Sci., v. 3, pt. 2, p. 111–144, pl. 8–9.

(17) ——— ———

 1913. The effect of partial sterilization of soil on the production of plant food. II. The limitation of bacterial numbers in soils and its consequences. *In* Jour. Agr. Sci., v. 5, pt. 2, p. 152–221.

(18) ——— and Petherbridge, F. R.

 1912. Investigations on "sickness" in soil. II. "Sickness" in glasshouse soils. *In* Jour. Agr. Sci., v. 5, pt. 1, p. 86–111, pl. 2–5.

(19) Wolff, Max.

 1909. Der Einfluss der Bewässerung auf die Fauna der Ackerkrune mit besonderer Berücksichtigung der Bodenprotozoen. *In* Mitt. Kaiser Wilhelms Inst. Landw. Bromberg, Bd. 1, Heft 4, p. 382–401, 49 fig.

TYLENCHUS SIMILIS, THE CAUSE OF A ROOT DISEASE OF SUGAR CANE AND BANANA

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OCCURRENCE OF TYLENCHUS SIMILIS IN FIJI AND HAWAII

A serious outbreak of a disease among bananas (*Musa sapientum*) in Fiji in 1890–91 caused the planters great uneasiness. At the request of Sir John Thurston, British High Commissioner of the Pacific, the Department of Agriculture of New South Wales, Australia, undertook an investigation, which was conducted by the writer. Most of the banana plants examined grew in the gardens adjacent to Government House at Suva, Fiji, where experimental plantings were made in connection with the disease. During the investigations roots of the banana and the soil about the roots were examined with a view to discovering possible causes of the disease. It was during this particular part of the investigation that a new species of nematode was discovered, to which the name "*Tylenchus similis*" was applied. Only the male was seen.

Nothing further was discovered concerning this species of Tylenchus until 1907, when, during a visit to sugar plantations on Kauai, one of the Hawaiian Islands, the same nematode was again found by the writer, this time infesting the roots of sugar cane (*Saccharum officinarum*). Both sexes of the nematode were found in abundance, and to these specimens, which at the time appeared to represent a new species, the name "*Tylenchus biformis*" was applied. *T. biformis* proved to be a true parasite and was found to be sufficiently injurious to the roots of sugar cane to justify a careful examination.

The nematode appeared to attack the roots at or near the tips, with the result that the root soon succumbed, thus compelling the plant to throw out new roots, which in turn became infested. The attacks of the nematode resulted in striking lesions, easily discoverable whenever the attacks were of a pronounced character. The tissues of the root lost their white or colorless appearance and took on first a cinnabar-red color, then a reddish purple color. The latter was succeeded by a dark purplish red, and this in turn by a purplish black. The discolored areas were sometimes several millimeters in length. In advanced cases the tissue of the axial part of the root was attacked, and large numbers of the nematodes were readily discovered in the colored cavities caused by their activities. The seriousness of the result was increased by the fact

that the breach created by the nematodes afforded entrance to fungous and microbic enemies.

It will thus be seen that this Hawaiian species of Tylenchus was found under circumstances conclusively proving its parasitic nature. Every degree of infestation was found in the sugar-cane roots, from those which upon external examination, even with a lens, appeared to be in a sound condition to roots spotted with numerous dark infested areas, each harboring scores of the nematode parasites. Sections of the roots showed that the cavities inhabited by the nematodes were colored or blackened on the inside and that it was this discoloration which gave rise to the outward appearances already described. All stages of the nematode were found in the cavities, including full-grown males and females, and it was plain that this species of Tylenchus lived generation after generation largely in the roots of the sugar cane, though it would undoubtedly be necessary, in the natural course of events, for the progeny sooner or later to remove from one root to another or from one plant to another. It was therefore to be expected that nematodes of this species would be found in soil adjacent to the roots of sugar cane, although the investigations made at the time did not disclose any stage of the parasite living free in the soil.

OCCURRENCE OF TYLENCHUS SIMILIS IN JAMAICA

Recently this nematode disease has been reported from the Island of Jamaica. The following are extracts from letters written by Mr. S. F. Ashby, Microbiologist of the Department of Agriculture, Jamaica:

I send you in a carton some fragments of diseased portions of rhizomes and true stems of the Jamaica (Gros Michel) banana preserved in dilute formalin. The disease, locally called "black head," shows as a black rot working into the tissue from the surface usually from around the insertions of diseased roots; the roots when attacked show depressed dark flocks at the surface, and within the cortex a purple rot disintegrating in the older parts.

The disease is widespread here owing to suckers for planting being frequently dug from affected stools; it is responsible for much backward growth and short bunches on land depending on rainfall in moderate or bad seasons.

Dr. Erwin F. Smith, after an examination of the material accompanying Mr. Ashby's letter, was of the opinion that the disease was not caused by *Fusarium* spp.

DESCRIPTION OF TYLENCHUS SIMILIS [1]

A comparison of the species of Tylenchus found in Hawaii with the other species known at the time seemed to indicate that it was not identical with any form previously described. It was, however, pointed

[1] For an explanation of the formula used in descriptions of nematodes *see* Cobb, N. A., Antarctic Marine Free-Living Nematodes of the Shackelton Expedition, p. 6, Baltimore, 1914 (Contrib. Sci. Nematology, I).

The illustrations were prepared under the author's personal supervision by Mr. W. E. Chambers.

out that the males and females were so unlike that, had they not been found in conjunction and under such circumstances as to preclude the possibility of error in assigning them to one and the same species, it is probable that they would have been considered to be separate species. The remarkable similarity of the male to those of the species of Tylenchus previously found about banana roots in Fiji did not escape notice, but as the Fijian observations were incomplete, no females of the Fijian species having been seen, the question of the identity of *Tylenchus similis* and *Tylenchus biformis* was not raised. The present investigation establishes the identity of these two species. The species should therefore bear the prior name "*Tylenchus similis* Cobb, 1892."

This nematode is, in the opinion of the writer, clearly proved to be the primary cause of a disease of the sugar cane. Mr. S. F. Ashby, in a recent letter, writes as follows concerning its relation to banana:

> I at first attributed the attack [on banana] to the joint action of a Fusarium and a coli-like bacterium frequently isolated from the rot; inoculation of either or both failed to cause a similar rot. Eelworms[1] were always found present, and on going through samples from various sources again I invariably came across the same species in the advance margin of the rot both in rhizomes and roots.

The specimens forwarded by Mr. Ashby contained no other organism that would appear to have caused the lesions.

The following description of *Tylenchus similis* is derived from specimens forwarded from Jamaica by Mr. Ashby in diseased banana tissues.

Tylenchus similis Cobb. *Tylenchus biformis* Cobb, 1907. $\left(\longrightarrow \dfrac{3.}{2.5} - - \dfrac{11.5}{3.2} - \dfrac{18.}{3.4} - \dfrac{-59.-^{38}}{3.8} - \dfrac{88.}{2.6} \right.$ .7 mm. The moderately thick layers of the transparent, naked, colorless cuticle are traversed by somewhat more than 400 transverse striæ, which are not further resolvable. The transverse striæ are interrupted on the lateral fields by conspicuous wings, the presence of which is indicated by four longitudinal striæ taking up a space equal to one-fourth to one-third the diameter of the body. The two outer of these lines are more conspicuous than the two inner, inasmuch as they are somewhat wider and more refractive. The two inner lines are sometimes faint and occupy about one-fourth to one-fifth the width of the entire wing space. The outer margins of the wings are almost imperceptibly crenate, a feature which is associated with the transverse striæ of the cuticle; the inner lines are also crenate, but even less markedly so. These wings begin opposite the base of the spear, where, however, they are not so pronounced as along the median regions, and extend backward to near the end of the tail. They maintain their maximum development in a rather uniform way from opposite the nerve ring to a little behind the anus. The posterior portion of the neck is subcylindroid, while the anterior portion is convex-conoid, and ends in a rounded head, which in the

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[1] Nematodes.

female has a flattish, hemispherical lip region, set off by a more or less distinct constriction. The striæ begin to diminish in size in the neighborhood of the base of the spear, and are only about one-third to one-half as wide at the base of the lips as they are farther back. These transverse striæ are so pronounced a feature that they give to the contour of the body a crenate appearance, especially toward the posterior extremity. The lip region also is minutely transversely striated, the number of labial striæ being about 8 to 10. There are arched radial ceratinous elements in the lip region, but these have not been accurately counted. It seems likely there are about six of them. The mouth opening is very small, and the vestibule is strengthened by ceratinous elements which serve as a guide to the spear. This latter is somewhat longer than the base of the head is wide and in the females at least is a strongly developed and doubtless very efficient organ. It may be divided into two regions the posterior of which is cylindrical, and ends at its hinder extremity in a strongly developed threefold bulb, about one-fourth as wide as the corresponding portion of the head, and to which are attached muscles that pass forward to near the outer portion of the base of the lip region. The anterior half of the spear is narrower, ends anteriorly in a somewhat blunt point, and is hardly half as wide as the larger posterior cylindrical portion. At the base of the spear the œsophageal tube begins. At this point it is about two-fifths to one-half the width of the corresponding portion of the neck. It has this diameter until near the median bulb, where it diminishes in such a way that at the actual junction with the bulb the diameter of the definite constriction separating it from the bulb is only one-fourth to one-sixth that of the neck. The median bulb is fairly well developed in the female, though much deteriorated in the male. In the female it is elongated to ellipsoidal in form, and about two-thirds as wide as the corresponding portion of the neck. It is supplied with a fairly well-developed but somewhat simple refractive valvular apparatus having a diameter nearly one-third as great as that of the bulb itself. Behind the bulb the œsophagus is again narrow—about one-sixth as wide as the corresponding portion of the neck. It soon widens out a little so as to become more than half as wide as the base of the neck. It joins the intestine in a somewhat indefinite manner. The length of the posterior part of the œsophagus may be judged by the fact that the distance from the anterior margin of the median bulb to the end of the œsophagus equals nearly half the length of the neck. In stained specimens the beginning of the intestine is indicated by the special cardiac cells of the intestine, which stain more strongly than the cells immediately behind them (fig. 1). The intestine is made up of cells which are packed with spherical granules of various sizes and of more than one kind. The smallest of the granules of the smaller sort have a diameter considerably less than the width of one of the striæ; the larger are two or three times as wide. The fatty granules or accretions of the intestine, the granules

of the larger sort, are of very much larger size and give to the organ its peculiar pearly appearance.

From the inconspicuous anus the rectum leads inward and forward a distance about equal to the anal body diameter. The tail is conoid to the rather blunt roughly conoid terminus, which has a diameter about one-third ot one-fourth as great as that of the base of the tail. On each lateral line, a little in front of the beginning of the middle third of the tail, there is a minute pore, which is possibly homologous with the single papilla found in the corresponding position on the tail of the male. The final striæ are rather indefinite, so that the terminus appears almost as if not striated. The lateral fields appear to be more than one-third as wide as the body. The excretory pore is rather conspicuous, as is the duct leading to it. Both walls of the duct are distinctly refractive, and its lumen may readily be seen. The pore is located about as far behind the median bulb as the base of the spear is in front of it. The duct leads backward a distance equal to three to four body widths, and there joins the rather small ellipsoidal renette cell located on the left-hand side of the body. The exact details of this renette cell are not yet clear. There is a conspicuous refractive cell of rather uniform granular texture located just behind the excretory pore. This cell is longer than the body is wide, about one-third as wide as long, and has a strongly refractive nucleus about one-fourth as wide as itself. Closely associated with this cell are two others of similar form but somewhat smaller, the three forming a close tandem series twice as long as the body is wide. As a rule, the two posterior cells of this series exhibit peculiarities not shown by the anterior cell; they do not stain so strongly with carmine, and in general are less conspicuous.

FIG. 1.—*Tylenchus similis:* Nearly adult female. *a*, Lip region; *b*, spear guide; *c*, 3-bulbed base of spear; *d*, ampulla, salivary gland; *e*, œsophageal lumen; *f*, œsophagus; *g*, median bulb; *h*, nerve cells; *i*, nerve ring; *j*, excretory pore; *k*, initial intestinal cells; *l*, anterior salivary gland; *m,m*, end of ovary; *n*, ovum; *o*, renette duct; *p*, posterior salivary gland; *q*, fat granule, intestine; *r*, renette cell(?); *s*, terminus; *t*, caudal pore; *u*, vulva; *v*, anus; *w*, crenate cuticle; *x, x*, spermatozoa.

These three glandular cells empty through a narrow duct which enters the base of the œsophagus in the rear of the nerve ring, passes through the median bulb, being diverted to pass around the central valve on its dorsal side, and extends thence onward to near the

base of the spear, where the duct enlarges to form a distinct, elongated ampulla, emptying into the œsophageal lumen immediately behind the base of the spear. From the somewhat broadly elevated but otherwise not very conspicuous vulva the vagina leads inward at right angles to the ventral surface fully halfway across the body, where it joins the two uteri, one of which extends forward and the other backward. In the females found infesting sugar-cane roots on the Island of Kauai, in Hawaii, the thin-shelled eggs were observed to be about twice as long as the body is wide and fully five-sixths as wide as the body. They begin segmentation before deposition. The blastomeres are rather coarsely granular.

Male formula. $\left(\!-\!\!-\!\!o \; \dfrac{2.4}{1.8} \;-\!\!-\; \dfrac{12.}{2.6} \;=\!\!=\!/ \; \dfrac{18.}{2.8} \;-\!\!-\; \dfrac{-M\,^{32}}{2.6} \;-\!\!-\; \dfrac{89.}{2.2}\right)$ .7 mm. The male differs in many important respects from the female, not only in the form of the tail end but in that of the anterior extremity as well. The neck of the male tapers rather regularly from the intestine forward, though it decreases rather more rapidly in diameter anteriorly, where it ends in a short, somewhat subcylindrical or hemispherical lip region set off by a deep and distinct constriction. This lip region appears to be composed of about the same number of striæ as that of the female, and to have the same general structure in spite of its difference in form and size. The spear of the male, however, is very weakly developed and is nothing like so efficient an organ as that of the female; in fact, at times it is difficult to convince oneself that the male really possesses an oral spear. From the structure of the mouth of the male it appears somewhat doubtful whether he is able to make his way unaided into the tissues of the host plant. It seems more probable that he works his way into the cavities already created by the voracity of the female. The bulbous base of the spear is no wider than one of the nearby annules of the cuticle, and the shaft at its widest part is considerably narrower than any of the annules of the cuticle. It tapers anteriorly to an excessively fine narrow point. The wings of the male are similar to those of the female, but are hardly so strongly developed (fig. 2). The tail tapers from some distance in front of the anus and diminishes in size rather regularly to near the blunt terminus. The posterior portion is subcylindroid and ends in a bluntly conoid terminus, which is about half as wide as the base of the tail, and which, like that of the female, is not provided with a spinneret. The bursal flaps spring from the submedian lines at a point just in front of the proximal ends of the spicula. When the body is seen in profile, the bursa extends beyond the ventral contour from opposite the proximal ends of the spicula to near the middle of the tail and continues almost to the end of the tail. Near the junction of the middle and anterior thirds of the tail there are two ventrally submedian, finger-shaped papillæ, which extend into the bursa and appear to reach about halfway to its margin. The bursa, like the cuticle, is striated, and its margin is crenate. The

regular striations of the cuticle extend nearly to the terminus.   The two equal, slightly arcuate, or nearly straight, tapering spicula are about one and one-third times as long as the anal body diameter.   Their proximal ends are cephalated by constriction.   At their widest part, which is near the constriction, they are about one-fifth as wide as the corresponding portion of the body.   Distally they taper to a slightly blunt point.   The accessory pieces are about half as long as the spicula and are placed parallel to them.   Passing inward the accessory piece increases in thickness for some distance and then near the middle begins to taper and at the same time to curve away from the spicula almost imperceptibly.   The accessory piece appears to have attached to it muscles which pass backward, but the distal attachment of these muscles has not yet been made out.   The single testis extends forward and has its tapering blind end located considerably behind' the middle of the body. The proximal portion of the testis is about one-half as wide as the corresponding portion of the body.   The males are more rare than the females, the ratio appearing to be about 1 male to every 5 to 10 females.

It is a rather remarkable feature of this species that the young have tails more blunt than the adults, the reverse being usually the case with nematodes.

FIG. 2.—*Tylenchus similis:* Male. *a*, Lip region; *c*, base of the spear; *e*, œsophageal lumen; *f*, œsophagus; *g*, vestige of bulb; *h*, nerve cell; *ı*, nerve ring; *j*, excretory pore; *l*, a nerve cell; *m*, end of testis; *q*, fat granule of intestine; *s*, terminus; *t*, bursal papilla; *v*, accessory piece; *x*, spermatozoa; *y*, left spiculum; *z*, bursa.

Habitat. (1) About the roots of bananas (Fiji); (2) in the roots of sugar cane (Kauai, Hawaii); (3) in the roots of bananas (Jamaica).

CONCLUSION

On the basis of our present knowledge it is impossible to suggest the original habitat of this nematode. In view of its habits, its known distribution indicates that it is adapted to tropical and subtropical conditions of widely different character. Its infestation of plants differing from each other so widely as banana and sugar cane leads to the suspicion that it may be another addition to the already formidable list of nematode parasites which adapt themselves to a great variety of conditions. Its presence in Jamaica suggests the possibility of its introduction thence into Porto Rico and the southern portions of the mainland of the United States, where it would probably find suitable host plants in the sugar cane and might be expected to attack other plants.

In one way this investigation of the anatomy of _Tylenchus similis_ adds materially to our knowledge of the group of Tylenchi to which it belongs. For a long time observers have noted in this group the presence of puzzling tissues or organs near the base of the neck, and these have been described and figured in a way that indicated a very incomplete and unsatisfactory knowledge of their real nature; in fact, they have always been regarded simply as constituents of the cardiac bulb. These researches prove that in _Tylenchus similis_ these peculiarities of the base of the neck are due to the presence of a threefold gland emptying through the lumen of the œsophagus near the base of the spear.

What appear to be homologous organs are known in other genera and are regarded as "salivary glands," admittedly more on the basis of their structure than on the results of physiological tests. However, the morphological evidence is very strongly in favor of the conclusions reached.

The presence of such organs has not hitherto been noted in Tylenchus or any nearly related genus. The details of the organ are difficult to follow, but once they had been demonstrated it became evident that a similar organ exists in other species of Tylenchus, and it is especially interesting to note the presence of a similar organ in the well-known _T. dipsaci_ Kühn, or, as it is yet more commonly known, _T. devastatrix_ Kühn, the devastating nematode, so often responsible in the past for great damage to bulbous crops, such as the onion and hyacinth. This similarity in structure between _T. dipsaci_ and _T. similis_ makes it all the easier on structural grounds to suspect _T. similis_ of becoming a serious pest whenever it gets an opportunity. Whatever may be the cause, there is no doubt of the ability of this species rapidly to break down the tissues of the plants it attacks. One may now suspect, and on very good grounds, that this ability is due not only to the battering action of the oral spear but to the chemical action of a special secretion. Entirely in accord with these ideas is the absence of this organ in the male of _T. similis;_ when the oral spear deteriorates, the gland deteriorates also.

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